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Aesculin-fermentative Coliform Bacilli and Diarrhea

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Introduction

Many studies have been reported on the biological properties of coliform bacilli. However, there has been little interested in aesculin-fermentation. Dr. Shikinami[1] (1951) reported upon the relation of aesculin-fermentative coliform bacilli with diarrhea as follows: aesculin-fermentative coliform bacilli were found in the feces of diarrheal patients at a higher rate than in those of healthy adults. The author investigated aesculin-fermentative coliform bacilli in the feces of newborn infants, adult diarrheal patients and healthy adults, and discussed whether such coliform bacilli are of clinical significance or not.

Experimental

Methods

Fresh feces were taken from infants 1 day to 75 days old, from adult patients suffering from chronic diarrhea for the past 1/2–1 years and from healthy adults, doctors and nurses working in our hospital. The feces were cultured in Endo's medium. Coliform bacilli thus cultured were separated and classified biologically on the basis of morphology. Biological examinations included Gram's staining, motility, carbohydrate-fermentative action, H_2S-production, urea-decomposing, ability to grow in the citrate medium, indole reaction, liquefaction of gelatine, coagulation of cow's milk, Voges-Proskauer reaction and methylred test. Serologically, the classification was made on the basis of the diagnostic coli antigenic schema according to Kauffmann, Knipschildt and Vahlne.[2] The positive aesculin-fermentation was determined by the disappearance of fluorescence after coliform bacilli were cultured in 1 per cent aesculin-bouillon at 37°C for 24 hours and by the appearance of black precipitate after adding 10

per cent $FeCl_3$ solution to the medium.

Results

The isolated coliform bacilli and aesculin-fermentation

From the feces of 44 newborn infants, 100 strains of coliform bacilli were isolated. Aesculin-fermentative coliform bacilli were found in 26 cases (59%), and aesculin-nonfermentative in 18 cases (41%). The determination of O antigen according to the diagnostic coli antigenic schema showed that aesculin-fermentative cases were 23 (88.4%), and aesculin-nonfermentative cases 8 (44.4%). Among these, K antigen was found in 15 cases (65%) in the former and 5 cases (62.5%) in the latter. In the classification of each O antigen, O–11 was found to be the highest rate in the former, and O–13 occurred rather frequently in the latter. Lactose-nonfermentative coliform bacilli, i.e., Paracolon, were observed in 11 cases (25%), but Arizona, Ballerup and Bethesda paracolon were not isolated. See Table I.

TABLE I

Experiments on 44 Newborn Infants

Aesculin-positive coliform bacilli—26 cases (59%)		Aesculin-negative coliform bacilli—18 cases (41%)	
Antigen	Number of positive cases	Antigen	Number of positive cases
O-5	5	O-13	3
O-6	2	O-15	2
O-7	2	O-17	1
O-9	3	O-20	2
O-11	7		8 (44.4%)
O-13	1		
O-15	2		
O-17	1		
	23 (88.4%)		
K	15 (65%)	K	5 (62.5%)

From the feces of 65 adult diarrheal patients, 121 strains of coliform bacilli were isolated, which were examined in comparison with those of the infants. Aesculin-fermentative coliform bacilli were found in 27 cases (41.5%), aesculin-nonfermentative in 38 cases (58.5%). O antigen could be determined in 18 cases (66.7%) in the former and in 20 cases (52.6%) in the latter. K antigen was found in 13 (72.2%) and 14 cases (69.9%) respectively. In the classification of each O antigen, each case of O–26 and O–111 (α coli) and two cases of O–55 (β coli) were found in the aesculin-positive cases. Paracolon were isolated in 14 cases (21.5%) and two of these belonged to Bethesda paracolon. These results are shown

in Table II.

TABLE II

Experiments on 65 Adult Diarrheal Patients

Aesculin-positive coliform bacilli—27 cases (41.5%)		Aesculin-negative coliform bacilli—38 cases (58.5%)	
Antigen	Number of positive cases	Antigen	Number of positive cases
0-1	1	0-1	1
0-5	2	0-3	1
0-6	1	0-4	2
0-7	1	0-5	1
0-12	2	0-6	2
0-14	2	0-9	1
0-19	1	0-13	5
0-23	2	0-14	1
0-25	2	0-15	3
0-26	1	0-17	1
0-55	2	0-20	1
0-111	1	0-24	1
	18 (66.7%)		20 (52.6%)
K	13 (72.2%)	K	14 (69.9%)

In 178 coliform strains isolated from the feces of 110 healthy adults' aesculin-fermentative coliform bacilli were examined. Aesculin-fermentative coliform bacilli were found in 16 cases (14.5%), and nonfermentative in 94 cases (85.5%). O antigen could be determined in 8 (50%) out of 66 positive cases and 16 (43.2%) out of 37 negative cases. K antigen was found in 6 cases (75%) in the former and 11 cases (68.7%) in the latter. In the classification of each O antigen, no significant difference was observed between the positive and negative cases. Paracolon were found in 17 cases (15.4%), but Arizona, Ballerup and Bethesda paracolon were not isolated. See Table III.

In the adult diarrheal patients, aesculin-fermentative coliform bacilli were examined in relation to the duration of diarrhea. In the treatment of three chronic diarrheal patients excreting aesculin-fermentative coliform bacilli, it was observed that in two cases aesculin-fermentative coliform bacilli disappeared in 1–2 months after the patients had recovered.

Aesculin-fermentation of Kauffmann's standard coliform bacilli

In 28 coliform strains from 0–1 to 0–26, besides 0–55 (β coli) and 0–111 (α coli), shown in the diagnostic coli antigenic schema according to Kauffmann, Knipschildt and Vahlne, aesculin-fermentative strains were examined. It was found that 53.5 per cent of them fermented aesculin after 24 hours and 71.4 per cent after 48 hours respectively. In the present experiments, it was noticed with interest that 0–55 (β coli) and 0–111 (α coli) fermented aesculin rapidly, which were reported as

TABLE III

Experiments on 110 Healthy Adults

Aesculin-positive coliform bacilli-16 cases (14.5%)		Aesculin-negative coliform bacilli-94 cases (85.5%)	
Antigen	Number of positive cases	Antigen*	Number of positive cases
0-2	1	0-1	2
0-5	2	0-6	1
0-6	1	0-7	1
0-13	1	0-9	1
0-16	1	0-12	1
0-20	1	0-13	5
0-25	1	0-16	3
	8 (50%)	0-23	2
			16 (43.2%)
K	6 (75%)	K	11 (68.7%)

* About 37 of 94 negative cases, 0 antigen was investigated.

the cause of infantile diarrhea or summer diarrhea in Europe.[3),4),5),6),7),8),9),10)] These results are stated in Table IV.

Aesculin-fermentation of Paracolon, Sallmonela and Shigella

Of various kinds of Paracolon given by the Kitasato Institute of Infectious Diseases in 1953, 17 Arizona, 21 Ballerup and 6 Bethesda strains were selected for the test of aesculin-fermentation. All Arizona strains were negative, and only one strain in Ballerup and 4 strains in Bethesda were positive.

Of 71 Salmonella strains in various groups shown in the Salmonella antigenic schema according to Kauffmann and White (1937), S. paratyphi A, S. paratyphi B, S. stanley and S. heiderberg fermented aesculin, but the others were mostly negative.

For Shigella, 15 strains belonging to various groups shown in the international Shigella antigenic schema were tested. Aesculin-fermentative strains were not found. See Table V.

The toxic test for mouse and oral administration to the human body

Differences in toxic effect on mice between aesculin-fermentative and nonfermentative coliform bacilli were tested. Aesculin-fermentative coliform bacilli i.e, 0–4, 3 L, (5) and 0–5, 4 L, 4 selected from Kauffmann's standard coliform bacilli, Sato strain (0–4) and Ando strain (0–9) isolated from the patients were examined in comparison with aesculin-nonfermentative coliform bacilli, i.e., 0–17, 16 L, 8 and 0–18, 14, Matsui strain (0–13) and Endo strain (0–23). Five mice, each from 8 to 10 g. in body weight were used in each test and various quantities (1–10 mg.) were injected intraperitoneally. No significant difference in the toxic effect was observed.

TABLE IV

Aesculin-fermentation in Kauffmann's Standard Coliform Strains

Group	Antigen			Aesculin-fermentation	
	O	K	H	24 h.	48 h.
1	1	1L	(7)	(−)	(+)
2	2	1L	4	(−)	(+)
3	3	2a, 2bL	2	(−)	(−)
4	4	3L	(5)	(+)	(+)
5	5	4L	4	(+)	(+)
6	6	2a, 2cL	1	(−)	(+)
7	7	1L		(−)	(−)
8	8	50	(9)	(+)	(+)
9	9	9L	12	(+)	(+)
10	10	5L	4	(+)	(+)
11	11	10L	10	(−)	(−)
12	12	5L		(+)	(+)
13	13	11L	11	(−)	(−)
14	14	7L		(−)	(+)
15	15	14L	4	(+)	(+)
16	16	1L		(+)	(+)
17	17	16L	18	(−)	(−)
18	18		14	(−)	(−)
19	19		7	(+)	(+)
20	20	17L		(+)	(+)
21	21	20L	(4)	(+)	(+)
22	22	13L	1	(+)	(+)
23	23	18L	15	(−)	(−)
24	24			(−)	(−)
25	25	19L	12	(+)	(+)
26	26	(H311b)		(−)	(+)
55	55	B5		(+)	(+)
111	111	B4		(+)	(+)

TABLE V

Aesculin-fermentation in Paracolon, Salmonella and Shigella

Bacilli		Nnmber of aesculin-fermentative strain
Paracolon		
Arizona	17 strains	0
Bethesda	6	4 (66.6%)
Ballerup	21	1
Salmonella	71	4 (5.6%)
Shigella	15	0

In the oral application of aesculin-fermentative coliform bacilli to the human body, it was examined whether such coliform bacilli, invading the intestinal tract, multiplied and caused to have diarrhea or not. Three strains of aesculin-fermentative coliform bacilli, i.e., 0–4, 3 L, (5) selected from Kauffmann's standard coliform bacilli, Sato strain (0–4) and Saito

strain (0–25) isolated from the diarrheal patients were taken and administered in quantities of about 2 mg. to three healthy men, respectively. No remarkable symptoms were observed.

Discussion and Conclusion

In the feces of infants, adult diarrheal patients and healthy adults, 59, 41.5 and 14.5 per cent of aesculin-fermentative coliform bacilli were found, respectively.

The diarrheal feces in newborn infants are attributable to the fact that filth or bacterium may be swallowed by them at the time of delivery, and the fact that the intestinal tract, which has been kept quiet in the mother's body, suddenly begins to move after birth. Probably the most important cause is the fact that, coliform bacilli, invading the sterile intestinal tract of newborn infants for the first time, multiply rapidly.[11] In the present experiments, aesculin-fermentative coliform bacilli were found in the highest proportion in the diarrheal feces of newborn infants.

In the adult chronic diarrheal patients aesculin-fermentative coliform bacilli were found in the next highest proportion, but these disappeared with considerable frequency as the patients became halthy.

In Kauffmann's standard coliform bacilli and Bethesda paracolon, aesculin-fermentative strains were frequently found, just as in the diarrheal feces mentioned above: 53.5 per cent of Kauffmann's bacilli and 66.6 per cent of Bethesda paracolon fermented aesculin, respectively. Among these, 0–55 (β coli) and 0–111 (α coli) which were reported as the cause of infantile diarrhea or summer diarrhea in Europe fermented aesculin rapidly.

From these experimental results, it is clear that aesculin-fermentative coliform bacilli are associated with diarrhea.

Classifying O and K antigen on the basis of the diagnostic coli antigenic schema according to Kauffmann, Knipschildt and Vahlne, no par ticular relation between aesculin-fermentative and nonfermentative coliform bacilli was recognized. And in the toxic test on mice, no significant difference was observed between the two types of bacilli. In the oral administration of aesculin-fermentative coliform bacilli to the human body, no remarkable effects were observed.

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Interaction of Successive Photic Stimuli in the Human Retina

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Introduction

Motokawa[1] found that the electrical excitability of the dark-adapted retina considerably increases a few seconds after an illumination of the retina, and based on this phenomenon a new method has been developed for studying the effect of the light upon the retina. In the experiment by Motokawa,[2] the electrical excitability of the eye after a white test light stimulus showed a remarkable change characteristic of a conditioning colored light, when this preceded the white test light. However, the effect of the test light as measured by electrical stimulation following it remained little altered when the conditioning stimulus was white light of about the same intensity as that of the test stimulus. The interval between the conditioning and the test stimuli was a few seconds, and their duration was 2 seconds.

Motokawa and Ebe[3] further observed that two flashes of white light successively presented at an interval of 90–170 msec. produced a color-effect as tested by Motokawa's method.[4] In other words, the effect of two flashes of white light is quite different from that of a single flash, when the interval between two flashes is adequate. These experiments indicate that a remarkable interaction takes place between two white light stimuli under certain experimental conditions, but not under others.

The present investigation was undertaken in order to specify more precisely the conditions for the interaction of two white light stimuli.

Experimental

Method

The effect of light was measured in terms of increases in electrical excitability following illumination. A quantitative expression for the effect is as follows: $\zeta = 100\ (E - E_o)/E_o$, where E and E_o represent electrical

excitabilities with and without pre-illumination. The method and procedure for measuring E and E_o are described in detail in the previous paper.[5]

The subject was dark-adapted for about 20 minutes before each experimental session. The light stimuli used were flashes of white light 40 msec. in duration. The intensities of the conditioning and the test lights were controlled separately by means of neutral filters.

An opaque glass disc of 0.5 cm. in diameter was illuminated from behind by white light and viewed from a distance of 30 cm., so that the visual angle subtended by the disc at the eye was 1°.

Results

The ζ-time curves for white light are illustrated in Fig. 1. In these experiments a retinal area of 20° from the foveal center was exposed to a single flash of white light 40 msec. in duration, and ζ values at various

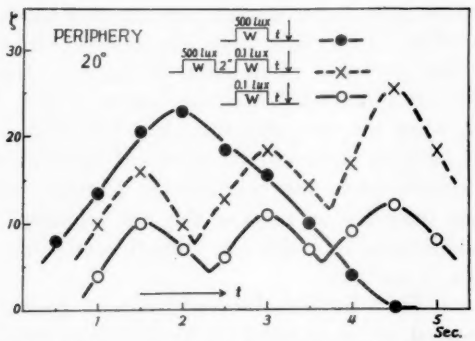

Fig. 1. ζ-time curves obtained at periphery 20° with illumination of 0.1 lux (empty circles) and 500 lux (solid circles). Broken curve was obtained by successive presentation of conditioning flash of 500 lux and test flash of 0.1 lux. Ordinates: Percentage increases of electrical excitability over resting level. Temporal sequence of procedure is shown in inset where W denotes a flash of white light 40 msec. in duration.

times after the flash were measured. The full curve connecting empty circles shows the ζ-time curve for a flash of 0.1 lux. We can distinguish three elevations in the curve. The elevation at 4.5 seconds after the flash represents the rod-process for the reasons stated by Motokawa *et al.*[6-8] The maxima of the other two elevations lie at 1.5 and 3 seconds respectively. According to Motokawa's analysis,[4] the elevation at 1.5 seconds represents the yellow process, while that at 3 seconds represents the blue process. The full curve connecting solid circles for a flash of

500 lux has a single maximum at 2 seconds, and no rod-process appears in the curve. The broken curve connecting crosses represents the result obtained, using white lights of 500 lux and 0.1 lux as the conditioning and the test flashes respectively. The two flashes were separated by an interval of 2 seconds. The broken curve is similar in shape to the curve for a single flash of 0.1 lux mentioned above. As can be seen in the broken curve, the magnitude of each process is much higher than the corresponding process for the test flash of 0.1 lux alone. In other words, the effect of the two stimuli is obviously greater than that of the single. Although the mechanism of this phenomenon is not known at present, it may be called " summation."

In order to study the effect of the intensity of the test flash on summation, the following experiment was performed at 20° from the fovea, fixing the intensity of the preceding flash at 500 lux or 10 lux. In Fig. 2, ζ values are plotted as ordinates against the logarithm of the intensity of the test flash in lux as abscissas. The magnitude of the rod-process in response to the test flash alone is represented by the full curve marked by empty triangles.

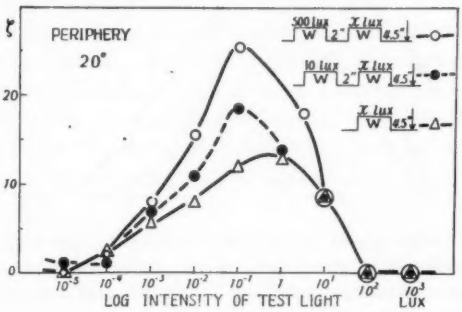

Fig. 2. The dependence of magnitude of rod-process upon the intensity of test flash for conditioning flashes of 500 lux (curve marked by empty circles) and 10 lux (curve marked by solid circles). Curve marked by empty triangles was obtained with test flash alone of various intensities.

In agreement with the data reported by Oikawa and Kurosawa[9-10], the rod-response increases in magnitude to some extent, as the intensity of illumination rises, but decreases above a certain intensity. Moreover, the rod-process is completely suppressed above 100 lux (inhibition of the rod-process). As is shown in Fig. 1, no rod-process appeared in the curve representing the effect of the 500 lux flash alone. The curve connecting empty circles represents the data of rod-responses obtained with varying intensities of the test flash and a fixed intensity of 500 lux of the con-

ditioning flash. The two stimuli were separated by an interval of 2 seconds. This curve is generally higher than the curve for the test stimulus alone, except for the intensity range higher than 10 lux. This means that summation takes place unless the test flash is too strong. The difference between the summation curve and the control curve increases gradually as the intensity of the test stimulus rises and attains a maximum at 0.1 lux. The broken curve marked by solid circles represents a summation curve obtained with a conditioning stimulus fixed at 10 lux. In this case no summation can be seen at intensities of the test flash higher than 1 lux. In this manner the intensity of the test stimulus has a great effect upon the summation.

In the next series of experiment, summation of cone-process was studied at the fovea. ζ-time curves obtained from the fovea are illustrated in Fig. 3. The full curve connecting empty circles was obtained

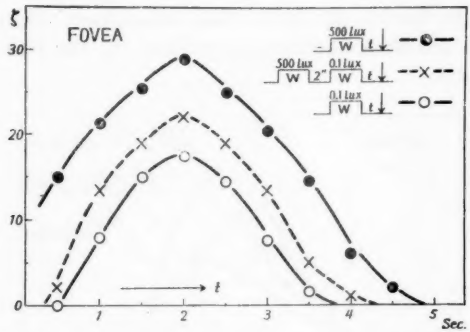

Fig. 3. ζ-time curves obtained at fovea with a single flash of 0.1 lux (empty circles) and of 500 lux (solid circles). Broken curve was obtained by two flashes of intensities 500 and 0.1 lux delivered successively at interval of 2 seconds.

by a single flash of 0.1 lux, and the one connecting solid circles by a single flash of 500 lux. Both curves show their maxima at 2 seconds, and the time to the maximum remains constant irrespective of the light intensities. The magnitude of the responses is, however, higher for 500 lux than for 0.1 lux. Next, the conditioning flash of 500 lux and the test flash of 0.1 lux were presented in succession at an interval of 2 seconds, and a summation curve such as the broken curve in the figure was obtained. As can be seen in this figure, the summation curve is situated between the curve for 500 lux and that for 0.1 lux, running parallel to both curves.

In Fig. 4, the intensity of the test stimulus was varied for the fixed conditioning stimuli of 500 lux and 10 lux. The interval of both stimuli

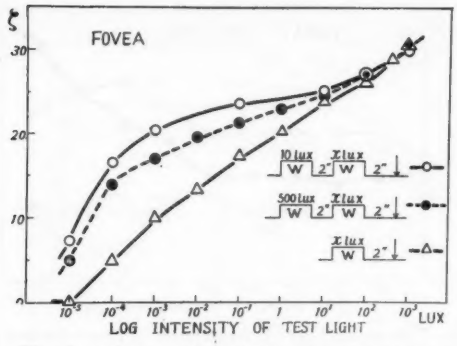

Fig. 4. The dependence of magnitude of cone-process upon the intensity of test flash. Intensity of conditioning flash was 10 lux for curve marked with empty circles and 500 for curve marked with solid.

was fixed at 2 seconds. The ordinates represent the magnitude of the cone-process at 2 seconds after the test flash and the abscissas the logarithm of the intensity of the test stimulus in lux. The full curve connecting empty triangles was obtained by the test flash alone. As indicated by this curve, the ζ values increase approximately in proportion to the logarithm of the intensity, so that no such depression at higher intensities as has been observed for the rod-process can be seen for the cone-process. The broken curve was obtained by successive presentation of the conditioning light of 500 lux and the test light of various intensities. Similarly the curve connecting empty circles was obtained, fixing the intensity of the conditioning stimulus at 10 lux.

As can be seen in this figure, considerable summation is seen in the range of lower intensities, but as the intensity of the test stimulus comes closer to that of the conditioning one, the magnitude of summation decreases, and no summation at all can be seen when the intensity of the test stimulus exceeds that of the conditioning one. This finding is in good agreement with the observation made by Motokawa[2] who found no summation whatsoever in his experiment carried out with two stimuli of about equal intensity. It is to be noted that the summation for the weaker conditioning stimulus is much greater than that for the stronger one; one might expect that a stronger stimulus would have a stronger after-effect.

In the following experiments the intensity of the conditioning stimulus, instead of the testing one, was varied. The data illustrated in Fig. 5 were obtained with the test stimulus fixed at 0.1 lux. The continuous and the broken curves represent the data concerning the rod- and the cone-processes respectively.

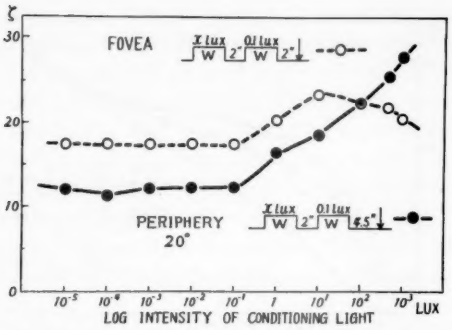

Fig. 5. The dependence of magnitudes of cone- (broken curve) and rod-processes (full curve) upon the intensity of conditioning flash, for test flash of 0.1 lux.

ζ values measured at 4.5 seconds after the test flash falling upon a retinal area 20° from the fovea were used as representation of the rod-process, and ζ values at 2 seconds in the fovea as the magnitudes of the cone-process. The summation curve for the cone-process (the broken curve) is flat and the magnitude of response is equal to that of the response to the test flash alone when the intensity of the conditioning stimulus is lower than that of the test stimulus. It is obvious that no summation is seen in this range of intensity. But summation occurs at higher intensities of the conditioning stimulus and is most conspicuous at 10 lux, that is, an intensity 100 times as high as that of the test stimulus.

In the rod-process (the full curve), the relation is the same as in the cone-process, that is, the conditioning stimulus weaker than the test stimulus causes no summation at all. The summation increases as the intensity of the conditioning stimulus rises above that of the test stimulus. There is, however, a remarkable difference between the cone- and the rod-processes; while for the summation of the cone- process there is an optimal intensity of the conditioning stimulus, there is none for the rod-process at least within the range of intensities tested so far.

In all the experiments mentioned above the interval between the two flashes was always 2 seconds. The interval was, however, varied between 0.5 and 25 seconds in the following experiments in order to study the dependence of summation on the interflash interval. The data are illustrated in Fig. 6, in which ordinates represent the ζ values at 2 seconds for the cone-processes and 4.5 seconds for the rod-process after exposure to the test stimulus respectively and abscissas the interval of the two stimuli in second.

The horizontal broken and continuous lines indicate the magnitudes

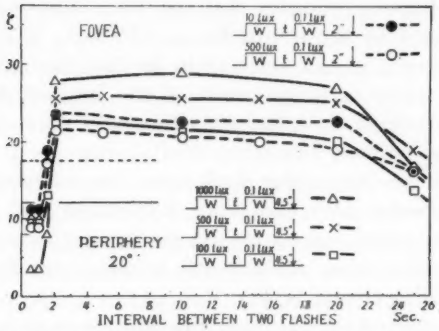

Fig. 6. Inhibition and summation as a function of interval between conditioning and test flashes. Broken and full curves represent magnitudes of cone- and rod-processes respectively. Horizontal broken and continuous lines represent magnitudes of cone- and rod-processes to test flash alone respectively.

of the cone- and the rod- processes for the test stimulus of 0.1 lux alone. The broken curves connecting solid and empty circles were obtained at the fovea with the conditioning flashes of 10 and 500 lux respectively, the test flash being 0.1 lux in both cases.

The data for the rod-process obtained at 20° from the fovea are represented by three continuous curves. The conditioning stimuli was 100, 500 and 1000 lux for each curve, the intensity of the test stimulus being 0.1 lux for all curves.

As can be seen in this figure, ζ values for two successive flashes are smaller than the values for the test flash alone as indicated by the horizontal lines when the interval is shorter than 1.5 seconds. It seems that a kind of refractoriness is set up by the preceding flash and continues as long as 1.5 seconds. After that, summation increases steeply as the interval between the two flashes is increased, and reaches a constant level at about 2 seconds. It is to be noted that summation lasts as long as 20 seconds or longer, and that this holds for not only the rod, but also the cone-processes. However the degree of summation as measured by the difference between the height of the summation curve and the corresponding horizontal line is generally higher for the rod-process than for the cone-process.

Discussion

As has been shown in Figs. 1 and 3, the ζ-time curves for the two flashes are of the same form as the curves for the test flash alone, but the magnitude of the former is generally greater than that of the latter (sum-

mation). This finding indicates that the time course of the summation curve is determined by the test flash.

Further evidence of this relation is the fact that the time course of the summation curve is independent of the interval between the two flashes. The preceding flash has an effect only upon the magnitude of the ζ-time curve, without any effect on its form.

The effect of the preceding flash upon the magnitude of the ζ-time curve may be divided into two types, facilitation and inhibition. The former has been called summation in the present experiments, and the latter was seen only when the interval between two flashes was shorter than 1.5 seconds, as shown in Fig. 6.

Another characteristic feature of our phenomenon is that summation takes place only with the conditioning flash much stronger than the test flash. This may be so interpreted that the after-effect of the preceding flash is generally so weak, especially with a weak conditioning light, that not only the shape but also the magnitude of the ζ-time curve is determined by the following test flash.

When the intensity of the conditioning flash is strong enough, the after-effect of this flash will modify the effect of the subsequent flash in two ways; when the interval between both flashes is too short, the light adaptation caused by the preceding one will make the subsequent flash less effective, so that the magnitude of the resulting ζ-time curve decreases (inhibition). When the interval is longer the effect of light adaptation will subside, and the other aspect of the after-effect—facilitation —will become manifest. The fact that facilitation can last as long as over 20 seconds reminds us of retinal induction as studied by Motokawa.[11]

According to this author, the intensity of retinal induction or a contrast effect, which means the difference between the effect of the conditioning and the test stimuli and that of the latter alone, depends solely upon the intensity of the conditioning stimulus, but independent of the intensity of the test stimulus.

In our experiments, however, the degree of summation is a function of the intensities of both conditioning and test stimuli. This property makes it difficult to identify our phenomenon with Motokawa's retinal induction.

Further studies will be necessary to clarify the essential nature of this phenomenon.

Summary

By the method of electrostimulation, the cone- and the rod-processes following two successive flashes of white light were separately investigated in the human retina.

1) The cone- and rod-responses to two flashes, conditioning and testing, are in shape almost identical with their responses to the testing flash alone, but the magnitudes of the responses are greater for the two flashes than for the single. This phenomenon is called summation.

2) For summation to occur, the intensity of the conditioning stimulus relative to that of the testing is important. No summation occurs at all when the conditioning stimulus is weaker than the testing.

3) When the interval between two flashes is shorter than 1.5 seconds, inhibition occurs, instead of summation. At intervals longer than 2 seconds the degree of summation is almost constant irrespective of the interval. Summation can still be seen at such a long interval as 20 seconds.

4) The degree of summation is greater for the rod-process than for the cone-process.

We are greatly indebted to Prof. K. Motokawa for his many valuable discussions and kind guidance.

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Experimental Studies on Cardiac Resuscitation

I. Studies on the Restoration of the Heart Rhythm in Ventricular Fibrillation

By

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Cardiac arrest is one of the most unfortunate accidents in surgical operation. This complication has been known since old days. Most of the victims died in a moment and were diagnosed as acute heart failure, status thymicolymphaticus, or death due to shock. No effective resuscitative methods were known. Recently, informations concerning cardiac arrest have increased with its frequent occurrence during operation on the organs in the chest cavity. During the past one year in the Surgical Service of Prof. Katsura, 3 cases of cardiac arrest were encountered among 760 operations, which were all directly connected with intrathoracic operations. Its frequency is 4.6% and a tendency of increase is foreseen. This investigation has been carried out in order to establish the most effective measure against this accident. The most important factor which decides the prognosis is the restoration of cardiac rhythm and prevention of cerebral damage. The resuscitation of cardiac rhythm after ventricular fibrillation has been studied, especially the factors of the time limit and several factors influencing the restoration of cardiac rhythm.

Experimental

Dogs were used for the experiments. After premedication with morphine chloride, intravenous anesthesia with pentobarbital was applied. The heart was exposed by thoracotomy under endotracheal positive pressure. The pericardium was widely opened. ECG was taken with a pen writing apparatus with occasional simultaneous writing of ECG with EEG by an 8 element EEG pen writing apparatus. Ventricular fibrillation was removed with an electric defibrillator made by us (Fig. 1). Ventricular fibrillaton was caused by a 5 volt current applied for 1/2 sec., and was removed by serial shocks under the following con-

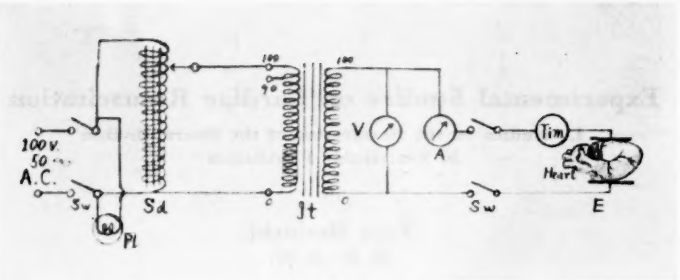

Fig. 1. Defibrillator circuit: Sd, slidac (500 watt. variable from 5 to 130 volt); It, isolation transformer (1:1); E, bipolar padded electrodes, (copper, 4 or 6 cm. diameter); T, timer (duration of stimulation 1/10 sec., interval of stimulation 1 sec.); V, voltmeter; A, amperemeter; Pl, pilot lamp; Sw, switch.

ditions; current 110 volt, duration 1/10 sec., interval between shocks, 1 sec. Cardiac massge was performed with firm grasp on both sides of the ventricle with the right thumb and the rest of the fingers, and blood was compressed out of the heart 50–60 times per minute.

I. Time Limit of Restoration of Heart Rhythm by Countershocks

A. Countershocks applied after various intervals

Method: Ventricular fibrillation of 20 canine hearts was abolished by electric shocks and the recovery of cardiac rhythm was observed.

Result: As shown in table I, if ventricular fibrillation was immediately removed within 1 minute by electric shocks, not a case ever failed to return to normal sinus rhythm. Arterial blood pressure once fell to zero during ventricular fibrillation, then rose rapidly above prefibrillatory level and gradually returned to normal height. Venous pressure rapidly rose during ventricular fibrillation, i.e., from 6 cm. to 20 cm. of water column; after electric shocks, it returned to normal level within 1 minute. Ventricular fibrillation was abolished by electric shocks within 2 minutes and normal cardiac rhythm was observed without external assistance. In some cases, recovery of cardiac rhythm was not so good and required cardiac massage for powerful contraction. It was possible to remove ventricular fibrillation by electric shocks within 3 minutes. Heart rhythm, however, was not powerful. Injection of 0.2 cc of 0.1% epinephrine into the left ventricle with cardiac massage was necessary. Neither arterial nor venous pressures returned to prefibrillatory level without heart massage and injection of epinephrine. Ventricular fibrillation could be removed by shocks after 3 minutes but the heart came to a standstill and the re-

TABLE I

Influences of Interval till Application of Electric Shocks on Ventricular Fibrillation without Cardiac Massage

Electric shock (E. S.); Ventricular fibrillation (V. F.); Cardiac massage (C. M.)

Lapse of time till E.S. (min.)	Effect of E.S. on V.F.			Restoring process of heart rhythm after removal of V.F.			Cases
	Removed by 1 shock	Removed by serial shocks	Recurrence of spontaneous V.F.	Within 1 min.	Over 1 min.	C.M. etc. required	
less than 1	14	0	0	14	0	0	14
1-2	14	2	2	11	2	5	18
2-3	4	2	0	1	1	4	6
more than 3	2	3	1	Normal heart rhythm not restored			
Total	34	7	3	26	3	9	44

covery of the heart rhythm was extremely difficult; as the heart muscles became flabby, only temporary effect could be expected by cardiac massage and injection of epinephrine. After removal of ventricular fibrillation, the ECG showed bad electric conduction, i.e., atrioventricular dissociation and slow wide QRS complex.

B. Electrical countershocks followed by cardiac massage

Method: In 14 dogs, heart massage was started immediately after ventricular fibrillation and after certain period of time, ventricular fibrillation was removed by electric shocks and the recovery of heart contraction was observed.

Result: As shown in Table II, ventricular fibrillation was immediately removed by countershocks in 20 minutes with restoration of normal cardiac rhythm. Blood pressure showed 80–90 mm. Hg. during cardiac massage and returned to preoperative level within 1 minute after its removal. When ventricular fibrillation was removed after cardiac massage was performed for 25–30 minutes, its removal became slightly difficult. The restoration of cardiac rhythm was prolonged and injection of epinephrine and additional cardiac massage were required. In many cases, arterial blood pressure rose with injection of epinephrine.

II. Restoration of Heart Rhythm by Countershocks against Ventricular Fibrillation during Hypoxia and Hypercapnea

A. Countershocks against ventricular fibrillation during obstruction of respiratory tract

TABLE II

Influence of Interval till Application of Electric Shocks on Ventricular Fibrillation with Immediate Cardiac Massage

Lapse of time till E.S. (min.)	Effect of E.S. on V.F.			Restoring process of heart rhythm after removal of V.F.			Cases
	Removed by 1 shock	Removed by serial shocks	Recurrence of spontaneous V.F.	Within 1 min.	Over 1 min.	C.M. etc. required	
5	1	0	0	1	0	0	7
10	1	0	0	1	0	0	1
15	3	0	0	3	0	0	3
20	2	0	0	2	0	0	2
25	2	1	0	0	0	3	3
30	0	1	1	0	0	2	2
Total	15	2	1	13	0	5	18

Method: In 12 dogs, the airway was obstructed for 1 minute followed by ventricular fibrillation by electric stimuli and left for 1 minute. Subsequently, countershocks were used to remove ventricular fibrillation with obstruction of the airway for 1–6 minutes. The obstruction was removed and artificial respiration performed with pure oxygen.

Result: As shown in Table III, ventricular fibrillaiton could be removed definitely and arterial blood pressure was restored to pre-opera-

TABLE III

Influences of Countershocks on Ventricular Fibrillation under Obstruction of Respiratory Tract

Obstruction of respr. tract (min.)	Effect of E.S. on V.F.		Restoring process of heart rhythm after removal of V.F.		Cases
	Removed by 1 shock	Recurrence of spontaneous V.F.	Within 1 min.	C.M. etc. required	
3	3	1	4	0	4
4	0	1	1	0	1
5	1	0	0	1	1
6	2	1	3	0	3
7	1	0	0	1	1
8	2	0	1	1	2
Total	9	3	8	4	12

tive level. Frequent occurrence of extrasystoles, however, followed and one fourth of the cases reverted to ventricular fibrillation. The recovery

of cardiac rhythm after removal of ventricular fibrillation in those cases which had release of obstruction of the airway within 4 minutes was strong. Massage was not needed, and powerful cardiac contraction was observed. In those cases with obstruction staying over 5 minutes, cardiac rhythm became powerful after removal of ventricular fibrillation at first. However, gradually bradycardia and standstill ensued. After the release of the obstruction, cardiac massage and injection of epinephrine were both required to strengthen the cardiac contraction.

B. Countershocks against ventricular fibrillation after release of obstruction of respiratory tract

Method: Using 9 dogs, after the airway was obstructed for 1 to 5 minutes, the obstruction was removed followed by ventricular fibrillation caused by electric stimuli for 30 seconds. Then countershocks were applied to the heart and the restoration of cardiac contraction was observed.

Result: As shown in Table IV, in those cases with obstruction of the respiratory tract up to 3 minutes, after removal of ventricular fibril-

TABLE IV

Influence of Countershocks on Ventricular Fibrillation soon after Release of Obstruction of Respiratory Tract

Obstruction of respr. tract (min.)	Effect of E.S.		Restoring process of heart rhythm after removal of V.F.		Cases
	Removed by 1 shock	Recurrences of spontaneous V.F.	Within 1 min.	C.M. etc. required	
1	2	0	2	0	2
2	1	0	1	0	1
3	1	0	1	0	1
4	1	1	1	1	2
5	1	2	0	3	3
Total	6	3	5	4	9

lation by countershocks, temporary tachycardia was observed. The heart, however, returned to normal sinus rhythm without cardiac massage. When the obstruction was applied over 4 minutes, after removal of ventricular fibrillation, the heart showed no recovery without assistance of cardiac massage. Ventricular fibrillation was induced by powerful cardiac massage in more than half of the cases. In such cases, if cardiac massage was performed 20 times per minute, ventricular fibrillation was not observed.

C. Countershocks against ventricular fibrillation soon after converting hypercapnia to pure oxygen breathing

Method: Dogs were made to breathe mixed gas of oxygen and carbon dioxide in various rates for 5–15 minutes followed by pure oxygen inhalation. Ventricular fibrillation was caused by electric stimuli and counter shocks were applied for 30 seconds. When O_2 gas mixed with CO_2 was sent, the blood pH fell from 7.00 to 6.80 with normal O_2 saturation. When artificial respiration was performed with pure oxygen, the pH level rapidly returned to the control level.

Result: When inhalation of high CO_2 concentration was changed to pure oxygen inhalation, most cases showed normal sinus rhythm except in a few cases with momentary scattered extrasystoles. As shown in Table V, it was more difficult to remove ventricular fibrillation than

TABLE V

Influences of Countershocks on Ventricular Fibrillation under Hypercapnia

Breathing gas (O_2:CO_2)	Removal of V.F. by E.S.		Restoring process of heart rhythm after removal of V.F.		Cases
	easy	difficult	within 1 min.	C.M. etc. required	
4:1	0	3	3	0	3
2:1	0	1	1	0	1
3:2	0	1	1	0	1
1:1	0	3	3	0	3
1:4	0	2	2	0	2
Total	0	10	10	0	10

in the aforementioned experiment. After removal of ventricular fibrillation, cardiac rhythm became forceful. Extrasystoles, however, frequently occurred and accompanied parasystoles. By injecting 0.4 mg. of 0.1% atropine, the extrasystoles decreased and normal sinus rhythm was recovered within a few minutes.

III. Countershocks against Ventricular Fibrillation Under Hypothermia

Method: After intravenous injection of 50 mg. of Hexamethonium bromide into the femoral vein of a dog, the body temperature was lowered to 25°C. after one hour and the chest was opened. Ventricular fibrillation was caused with electric stimuli and removed 30 seconds later. 120 minutes was required to elevate the temperature to preoperative con-

dition after closure of the chest.

Result: When the temperature of a dog was lowered, both the pulse and the respiration became slower, & normal respiration stopped at about 25°C. and bradycardia occurred. Though oxygen saturation was normal, the blood pH lowered slightly with a tendency of hypopotassemia. EEG from the cortex of the cerebrum was flat, while normal potential was observed in the spinal wave (ref. Fig. 2). With electric stimuli of 5 volt

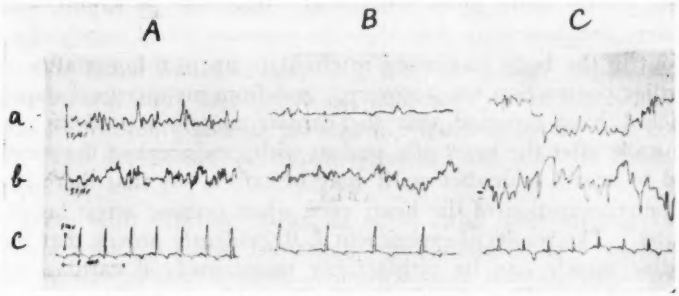

Fig. 2. ECG (lead II) (c); EEG (from cerebral cortex) (a) and spinal wave (b) during hypothermia experiment. A, preoperative control tracing; B, traced after 70 min, body temperature 25°C., EEG showing no potentials and ECG bradycardia; C, traced 120 min. after removal of ventricular fibrillation by countershocks, body temperature 36° C.

for 1/2 second, standstill of the heart occurred. In such conditions, ventricular fibrillation occurred following cardiac massage. Countershocks were not effective. Several attempts of cardiac massage followed with countershocks finally restored cardiac rhythm which had been almost completely in abeyance. When 1 cc. per kilogram of body weight of 5% KCl was injected into the heart, cardiac standstill occured. Following injection of 1.3 cc. of 5% $CaCl_2$ per kilogram of body weight, cardiac rhythm occurred only to change either to ventricular flutter or to ventricular fibrillation. After removal of ventricular fibrillation, cardiac rhythm recovered with elevation of body temperature, and normal spontaneous respiration with potential of brain waves from the cerebral cortex and normal sinus rhythm of heart contraction were observed.

Discussion

It is well known that ventricular fibrillation can be removed by serial electrical countershocks. The recovery of cardiac rhythm, however, has a definite limit in itself. As shown in the abovementioned experiment, ventricular fibrillation can be immediately abolished if it

is removed within 1 minute. In those cases with ventricular fibrillation lasting over 3 minutes, i.e., ventricular fibrillation in the fourth stage according to Wiggers' classification[1], they can be abolished by countershock, but in many cases effective cardiac contractions was not recovered. I agree with Dow and Wiggers[2] who claimed that, based on the same kind of experiments, if complete anoxia lasts over 1 minute, effective coordinated contraction of cardiac muscle can not be restored. The time limit of restoration of effective cardiac contraction which had undergone complete anoxia seems to be 3 minutes. According to Kountz's famous experiment demonstrating that when the coronary blood vessels of a heart taken out of the body was made to circulate up to 6 hours after death, the cardiac contraction was recovered, and from my personal experience of which I have reported that the cardiac muscle started to contract rhythmically after the heart of a patient with carcinoma of the esophagus stopped to beat 3 hours before, it may be safe to say that there is still a room for resuscitation of the heart even when cardiac arrest lasted over 3 minutes. The results of experiment *I, B.* evidently proves that the beat of cardiac muscle can be satisfactorily maintained, if cardiac massage is performed soon after ventricular fibrillation, and cardiac resuscitation is possible by removal of ventricular fibrillation by countershocks even if 20–30 minutes has lapsed.

It is considered that one of the most important mechanism of cardiac arrest is excessive sensitivity of the vagus nerve. Anoxia with hypercapnia during operation and frequent occurrence of hypercapnia under open chest are several factors influencing the tones of vagus. Experiment *II* was performed to understand the effect of countershocks against ventricular fibrillation under this condition. After removal of ventricular fibrillation during and after anoxia with hypercapnia, frequent occurrence of ventricular fibrillation was encountered (Experiment *II*, *A* & *B*). It was also difficult to remove ventricular fibrillation after removal of hypercapnia by countershocks (Experiment *II*, *C.*). As parasystoles occurred after the removal of ventricular fibrillation and as its removal is possible by injection of atropine, it may be said that the existence of hypercapnea increases vagal tone and enhances the irritability of cardiac muscle which is sensitive in causing ventricular fibrillation and subsequently it is difficult to remove ventricular fibrillation by countershocks. When anoxia existed over 4–5 minutes (Experiment *II*, *A* & *B*), cardiac contractions were weak after its removal and required cardiac massage and injection of epinephrine, while, in the case of hypercapina, cardiac pulsation was also weak after removal of ventricular fibrillation. From these facts, anoxic anoxia causes anoxia of the heart muscle and coordinated contractions of the heart muscle is interfered, while hyper-

capnia has no relationship to cardiac muscle. This suggestion is supported by the experiment by Young *et al.*[4] who showed that hypercapnia increased the effects of vagal stimulation, while hypoxia decreased its effects. Miller *et al.*[5] proved experimentally that when high alveolar carbon dioxide tension in cases of hypercapnea is converted suddenly to normal carbon dioxid tension, ventricular fibrillation occurred. They insisted the important role of hypercapnia as the cause of postoperative shock. Though ventricular fibrillation was not recognized during our experiment, its danger can be easily imagined from the above mentioned findings. If additional factor to hypercapnia occurs, conversion to ventricular fibrillation would be easy. In 1951, the author emphasized from an experimental study that circulatory collapse is apt to supervene during bilateral thoracotomy and its prevention should be given close consideration.[6] The prevention of hypercapnea should also be given close attention.

I agree with Bigelow, Swan, *et al.*[7),8] who have already reported that countershocks against ventricular fibrillation under hypothermia is not as effective as that applied at normal body temperature. Injection of KCl-$CaCl_2$ for the removal of ventricular fibrillation, as advocated by Swan *et al.*, however, did not prove to be superior to countershock. Fleming,[9] coworker of Bigelow, claims that prevention of a drop of pH decreases incidence of ventricular fibrillation. I have made the same kind of observations. The most decisive method to remove ventricular fibrillation might be countershocks and rapid rewarming will prevent it more definitely.

Summary

Experimental study was carried out to observe the mechanism of return of cardiac rhythm after defibrillation of a fibrillating heart caused by electric stimuli, with following results:

1. Cardiac rhythm is restored by countershocks if applied within 3 minutes.

2. As hypercapnia increases, the irritability of cardiac muscle increases also. Ventricular fibrillation is hard to remove by countershocks and reccurs also easily after its removal.

3. As anoxia weakens the contractility of cardiac muscle, resuscitation of the heart becomes difficult.

4. Ventricular fibrillation under hypothermia is difficult to remove by countershocks. Electric shock, however, seems to be the most effective method for its abolition.

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Experimental Studies on Cardiac Resuscitation

II. Studies on Resuscitation of Cardiac Arrest Caused by Various Conditions

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Among the various causes of acute cardiac arrest encountered daily, such as electrocution, vago-vagal reflex, mechanical stimuli of the heart and percardium, asphyxia, coronary occlusion, cardiac dysfunction due to oversupply of acetylcholine or epinephrine, hemorrhage, improper administration of anesthetics, etc., the first three causes can be obviously prevented by countershocks and massage as demonstrated from the experimental results described in my first report. Cardiac arrest, however, caused by the latter five accidents are due to complicated conditions. It is natural that the method of resuscitation should be different from the first three causes. As our knowledge is poor concerning the latter conditions, the method of resuscitation was studied experimentally as described in this account.

Experimental

I. Cardiac Arrest Caused by Obstruction of Respiratory Tract

Method: 11 adult dogs were used. Cardiac arrest was caused by obstructing the intubated endotracheal tube. Resuscitation was attempted by release of obstruction.

Result: After obstructing the respiratory tract, the lung became atelectatic within 3 minutes and the pulse showed transient gallop rhythm with temporary rise of blood pressure and changed to bradycardia. The cardiac rhythm became weak and a standstill was noticed 8–13 minutes later. When the obstruction of the respiratory tract was relieved and energetic artificial respiration with 100% oxygen was attempted with cardiac massage, the cardiac tonus was good and the pulsation gradually improved. Frequent appearance of extrasystoles and atrioventricular dissociation, however, appeared and changed into ventricular fibrillation in many cases. Many cases of ventricular fibrillation were incited

TABLE I

Cardiac Arrest Caused by Long Obstruction of Respiratory Tract

Duration of obstruction of airway (min.)	State of cardiac arrest	Time till restoration of heart beat after release of airway	Complication during cardiac resuscitation	Remark	Method of resuscitation
8	Standstill	6	V.F.	revived	1) Release of obst. & pure O_2 insuffl.
9	"	3	Rupture of R. Vent.	"	2) C.M.
10	"	19	none	"	3) E.S.
10	"	6	none	"	4) Injection of epinephrine (8 cases)
11	"	6	V.F.	"	5) Injection of procaine amid(7 cases)
13	"	10	"	"	
13	"	12	"	"	
14	"	8	"	"	
14	V.F.	7	Rupture of R. Vent.	died	
15	Standstill	10	V.F.	revived	
15	"	30	"	died	

after cardiac injection of epinephrine. In this case, ventricular fibrillation could be hardly removed by single use of countershocks. Injection of 50–100 mg. of procain amide into the heart followed by countershocks was frequently found to be efficient.

During powerful cardiac massage, the right ventricle was ruptured by manual pressure of the right thumb in two cases. All cases survived which had obstruction of the respiratory tract removed within 13 minutes.

II. Cardiac Arrest Caused by Ligation of Coronary Arteries

Method: As shown in Table II, the coronary arteries were dissected and only ligated at different points of the coronary arteries in 32 cases. In the cases of A, B & C, ventricular fibrillation was recognized within 2–8 minutes; In D & E, spontaneous occurrence of ventricular fibrillation was not observed for 20 minutes. Ventricular fibrillation was caused electrically and removed later.

Result: In the groups A, B & C, ventricular fibrillation could not be removed by mere countershocks. They were resuscitated by cardiac massage followed by countershocks after the release of the ligature. Fig. I shows the electrocardiographic findings in the group C. Generally speaking, ventricular fibrillation caused by the ligature at D & E was removed by countershocks. 1/3 of ventricular fibrillation at D, however,

TABLE II

Cardiac Arrest Caused by Ligation of Coronary Artery

(spont.), sponteneous occurrence of V. F.; (elect.), V. F. caused by E. S.

Group	Ligated portion of coronary artery	Cases	Time from ligation to occurrence of V.F. (min.)	Remark	Method of resuscitation
A.	Origin of left coronary artery	1	2 (spont.)	died	
B.	Origin of circumflex branch	5	2-5 (spont.)	revived	1) Release of ligat. 2) E.S. 3) Inhalation of O_2
C.	Origin of ant. descend. branch	9	2.1/4-8 (spont.)	"	
D.	Midportion of ant. descend. branch	13	(elect.)	"	1) E.S. 2) Inhalation of O_2
E.	Origin of right coronary artery	4	(elect.)	"	

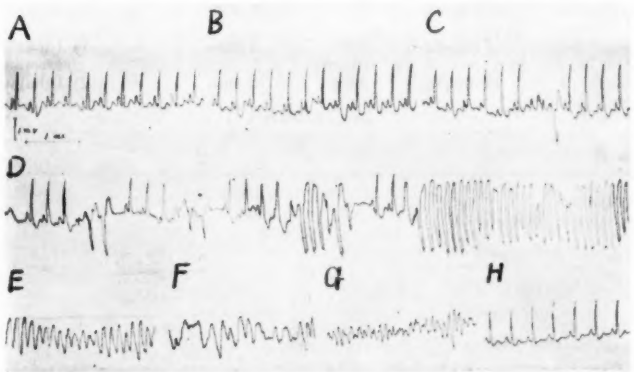

Fig. 1. ECG: Ventricular fibrillation caused by ligation of origin of ant. descend. branch of left coronary artery, lead II: A. preoperative control tracing; B. tracing 2 min. after ligation, ST segment depression; C, 3 1/2 min. initiation of premature beat; D, 5 min., transfer to ventricular fibrillation; E, tracing 1 min, after fibrillation; F. 2 1/2 min.; G, cardiac massage 3 min, after fibrillation; H, 2 min. after removal of V. F. by release of ligature with countershocks.

showed frequent occurrence of extrasystoles after removal of ventricular fibrillation. In many cases the heart became rumbling and started to fibrillate. In order to resuscitate the heart, the ligature had to be released. One of the cases ligated at E, countershock alone did not remove the fibrillation. Injection of 100 mg. of procaine amide followed by countershocks proved to be satisfactory. At first, ECG showed rise of

the ST segment and showed monophasic curve with gradual return to normal curve by massage.

III. Cardiac Arrest Caused by Acetyl Choline Injection

Method: 4 dogs were used. Cardiac arrest was caused by intravenous injection of acetyl choline (7–14 mg./kg.) and cardiac resuscitation was attempted.

Result: As shown in Table III, cardiac standstill occurred 5–8

TABLE III

Cardiac Arrest Caused by Injection of Acetyl Choline

Case No.	Body weight (kg.)	Dosis (intravenous injection)	Time from inj. till cardiac standstill	Time till restor. of heart beat	Remark	Method of resuscitation
108	10	8 mg./kg.	5 sec.	3 min.	revived	1) C.M. 2) Inhalation of O_2
109	6.5	7 mg./kg.	8 sec.	9 min.	"	
110	12	7 mg./kg.	6 sec.	3 min.	"	1) Injection of atropine
111	9	14 mg./kg.	5 sec.	8 min.	"	2) Inhalation of O_2

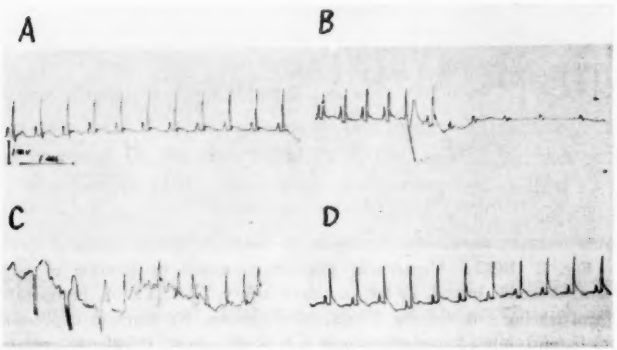

Fig. 2. ECG: Cardiac arrest caused by acetyl choline injection, lead II: A. preoperative control tracing; B, tracing 5 sec. after injecting 80 mg. acetyl choline, transfer to cardiac standstill; C, 1 min. after starting of cardiac massage; D, 3 min, after starting massage, sinus rhythm.

seconds after intravenous injection of acetyl choline. The tone of cardiac muscle was good. Several strong cardiac contractions appeared by cardiac massage at first and were followed with normal cardiac contractility 3–9 minutes later. Fig. 2 shows the ECG of case 10. This kind of cardiac standstill responded to intracardiac injection of 5 mg. of

atropine and rapidly returned to normal sinus rhythm.

IV. Cardiac Arrest Caused by Acute Massive Hemorrhage

Method: 5 dogs were used. A glass canule was inserted into the femoral artery and blood was withdrawn to the extent of 23 cc.–58 cc. pro kilogram of body weight till cardiac arrest occurred. Following this procedure, intraarterial transfusion was immediately performed with strong cardiac massage and cardiac stimulants such as epinephrine and vitacamphor were injected to resuscitate the heart.

Result: As shown in Table IV, in the case of massive blood loss, most cases went into cardiac standstill within 30 minutes. All cases showed flaccid cardiac muscle and died in spite of massage and injection of epinephrine etc.

Fig. 3 shows the ECG and EEG findings in case 116; 2'30" after blood was withdrawn: amount of blood loss 28 cc./kg. blood pressure 60 mm. Hg. ECG: lowering of ST segment & tachycardia. EEG: slow waves. Forced respiration started. 6': amount of blood loss 45 cc./kg. blood pressure, 20 mm. Hg., slight amount of bleeding, strong heart murmur audible. EEG: cerebral potential almost disappeared. 14': amount of blood loss 58 cc./kg., heart

TABLE IV

Cardiac Arrest Caused by Rapid Blood Loss

Cases No.	Body weight (kg.)	Volume bled	Time from begining to cardiac standstill (min.)	Remark	Method of resuscitation
112	14	28 cc./kg.	34	died	1) Intra-arterial transfusion
113	10	23 cc./kg.	28	"	
114	6	62 cc./kg.	36	"	2) C.M.
115	8	31 cc./kg.	31	"	3) Injection of epinephrine, $CaCl_2$ & $BaCl_2$
116	10	58 cc./kg.	30	"	

murmur not audible, pulsation hardly felt. ECG: sinus rhythm (Fig. 3, B). 16': spontaneous respiration stopped, pupil completely dilated. 20': cardiac fibrillation. 21': intraarterial transfusion with cardiac massage. 29': transfusion 60 cc./kg., blood pressure during cardiac massage 0–20 mm. Hg: tonus of the heart muscle slightly improved by injection of epinephrine and calcium chloride. 46': ventricular fibrillation removed by countershocks (Fig. 3, D.). 60': cardiac tonus not recovered and death.

V. Cardiac Arrest Caused by Rapid Intravenous Injection of Barbiturates

Method: 7 dogs were used. Cardiac arrest was caused by rapid

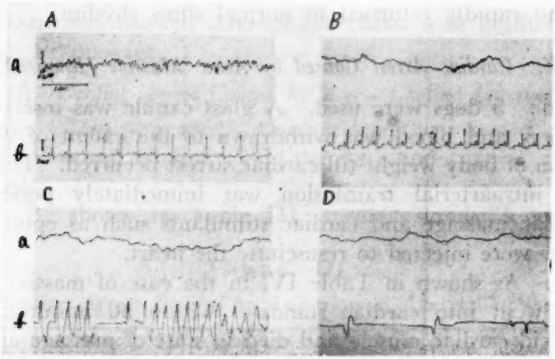

Fig. 3. EEG (a) ECG (b): Cardiac arrest caused by hemorrhage: A. preoperative control tracing; B, 14 min. after hemorrhage, EEG flat; C, 20 min, after hemorrhage, ECG transfer to vent. fibrillation; D, 46 min., fibrillation removed by E. S., ECG automatic by lower center.

intravenous injection of 30–50 mg. barbital sodium. Subsequently, cardiac resuscitation was attempted.

Result: As shown in Table V, cardiac standstill occurred within 5 seconds—6 minutes after intravenous injection. Immediately afterwards, cardiac massage was performed. Cardiac muscle, however, was flabby and soft. Blood did not circulate through the coronary vessels and cardiac rhythm could not be restored. The effect of epinephrine was only temporary and all cases proved fatal eventually. Case 121 showed strong cardiac contraction by cardiac massage. Respiration and brain stem reflex, however, did not appear and it died 120 minutes later.

TABLE V

Cardiac Arrest Caused by Rapid Injection of Barbital-Sodium

Case No.	Body weight (kg.)	Drug	Dosis (intravenous injection)	Time from inj. to card. stand.	Remark	Method of resuscitation
117	7	pent. barbit	43 mg./kg./30 sec.	6 min.	died	1) C.M. 2) Injection of epinephrine, $CaCl_2$ & $BaCl_2$
118	15	"	34 mg./kg./ 1 min.	5 sec.	"	
119	12	"	40 mg./kg./30 sec.	5 min.	"	
120	9	"	50 mg./kg./30 sec.	3 min.	"	
121	8	methyl-hexa-barbit.	50 mg./kg./20 sec.	4 min.	"	
122	10		40 mg./kg./15 sec.	2 min.	"	
123	11	"	30 mg./kg./10 sec.	2 min.	"	

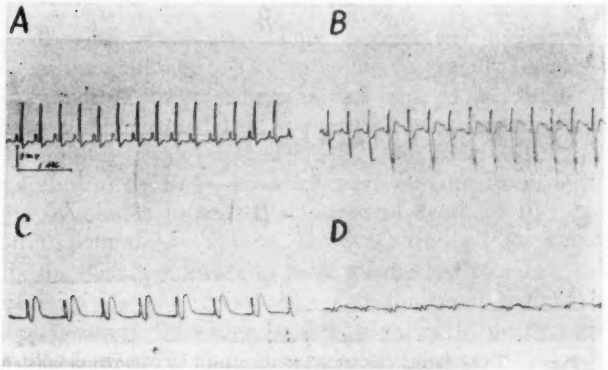

Fig. 4. ECG during cardiac standstill caused by intravenous injection of pentothal-sodium: A. preoperative control tracing; B, 10 sec. after injection 43 mg./kg. in 30 sec., deep S; C, 6 min., disappearance of P wave, low voltage of R, large T; D, 30 min. after massage; massage. injection of epinephrine, $CaCl_2$ & $BaCl_2$ ineffective.

Fig. 4 shows the ECG findings in case 117.

VI. Cardiac Arrest Caused by Chloroform Inhalation

Method: 5 dogs were used. Chloroform was either injected into the trachea in doses of 0.2–0.3 cc. pro kilogram of body weight, or in sufflation of large amount chloroform was rapidly applied with an anesthetic appratus, to cause cardiac arrest, and cardiac resuscitation was attempted.

Result: As shown in Table VI all cases showed cardiac standstill 20–30 seconds after the begining of inhalation of chloroform. After the start of cardiac massage, all cases showed ventricular fibrillation. After the start of cardiac massage, all cases showed ventricular fibrillation. They were temporarily removed by injection of procaine amide followed

TABLE VI

Cardiac Arrest Caused by Chloroform Inhalation

Case No.	Body weight (Kg.)	Method	Dosis	Time till card. stand.	Remark	Method of resuscitation
124	9	Intratracheal inhalation	0.2 cc./kg.	25 sec.	died	1) C.M. 2) E.S. 3) injection of epinephrine, $CaCl_2$ & procaine amide
125	6	"	0.3 cc./kg.	32 sec.	"	
126	12	anesthetic cap	?	60 sec.	"	
127	15	intratracheal	0.2 cc./kg.	30 sec.	"	
128	8	"	0.3 cc./kg.	20 sec.	"	

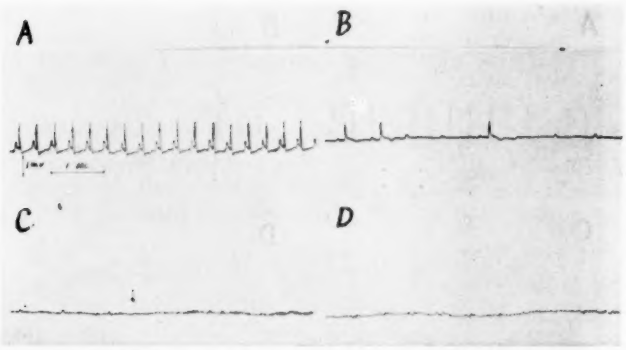

Fig. 5. ECG during cardiac standstill caused by chloroform inhalation. A, preoperative control tracing; B, 30 sec. after inhalation, abrupt disappearance of QRS complexs, prolongation of P-P interval; C, 40 sec., transfer to auricular flutter (arrow); D, 10 min., in spite of removal of V. F. by E.S., massage, injection of epinephrine etc. were ineffective, no vigorous heart beat restored.

by countershocks. Ventricular fibrillation, however, supervened again. Cardiac muscles were completely flabby and soft. Blood pressure did not rise by cardiac massage. Injection of epinephrne was not only ineffective, but excited ventricular fibrillation. All cases died eventually. Fig. 5 shows the ECG of Case 125.

Discussion

Cardiac arrest due to tracheal obstruction frequently occurs clinically such cases as intratracheal aspiration of vomitus, blood, tracheal obstruction due to sputum, laryngeal spasm, etc. Their prognosis, however, are relatively good. When the obstructon of the trachea is removed and assisted respiration by 100% oxygen and energetic cardiac massage are performed, cases with the tracheal obstruction released with in 13 minutes recover rapidly. When bradycardia develops during tracheal obstruction, section of the left vagus nerve causes tachycardia.[1] This phenomenon is due to increase of vagus irritability.

It is evident by my experiment and Wigger's experiment[2] that ventricular fibrillation following coronary insufficiency can only be cured by release of the causative factor, i.e., removal of occulusion and recovery of cardiac ischemia. Ventricular fibrillation due to occlusion of peripheral branches of the coronary artery and the right coronary artery, however, can be removed by countershocks alone. Administration of procaine amide with performance of cardiac massage at the same time, however, is much better.

Cardiac standstill due to oversupply of acetyl choline is attributed to increase of vagus irritability. This responds well to injection of atropine, and cardiac massage. The prognosis is usually good. All cases of cardiac standstill by bleeding turned out to be fatal. Negovski, Winkelbauer *et al.*[3),4)] stated that if cardiac resuscitation can be started within 5–6 minutes from "clinical death," the heart will recover. The standard of clinical death is, however, very obscure from my study. It seems to be reasonable to set the standard of death at the disappearing time of brain potentials. This is, however, difficult for clinical application. As the heart continues to beat till the last moment, the changes of ECG findings are not remarkable, and the heart is hard to recover once it stops beating. Measures against bleeding to death are to watch the fall of blood pressure, change of pulse and amount of blood loss, and to control the blood transfusion.

Cardiac arrest due to narcotics, i.e., barbiturates, and chloroform have bad prognosis. The muscles of an arrested heart are flabby, and neither massage nor injections of epinephrine, calcium chloride, cedilanid, and barium chloride are effective. Despite temporary return of pulsation, death comes on with disappearance of respiration and reflexes. As this kind of cardiac arrest is due to intoxication of heart muscle, brain and other vital organs, the prognosis is influenced by the amount of administration and speed of injection of the drugs. Therefore, the measure against this catastrophe is to select narcotics carefully and to prevent the accident beforehand by avoiding over-administration of the drugs. If possible, measures for detoxication is important.

Resuscitation of the arrested heart due to chloroform has been carried out many times since the first experiment by Schiff (1874). Chloroform, however, is abandoned as a narcotic at present time and became one of the classical drugs. One of its causes may be due to its difficulty to resuscitate the arrested heart as described above.

Beck *et al.*[5)] stated that ventricular fibrillation were apt to be caused by localized ischemia or anoxia of the heart, while cardiac standstill was brought about by generalized ishemia or anoxis. My experiment gave the same results. Except ventricular fibrillation caused by coronary occlusion, the cases showed cardiac standstill with few exceptions. Ventricular fibrillation occurred when epinephrine was injected within a heart under cardiac standstill due to obstruction of the trachea and inhalation of chloroform. On the contrary, in spite of intravenous injection of large dosage of epinephrine into a normal beating heart, the heart became rumbling but it was never thrown into fibrillation. From this finding, epinephrine acts as a secondary stress upon a very sensitive heart and excites ventricular fibrillation. The mechanical stimuli of

cardiac massage also acts as a secondary stress and induces ventricular fibrillation. When cardiac massage is required, it should be done gently during the refractory phase of cardiac muscle, i.e., the systolic phase to avoid unnecessary stimulation of the heart muscles. I should like to call this manner "*assisted massage.*" In such cases, as ventricular fibrillation is hard to remove by countershocks, it is desirable to use countershocks after cardiac sensibility is reduced by administration of procaine amide as shown by the experiment of Mautz *et al.*[6]

Summary

Cardiac arrest frequently encountered in routine treatments was caused experimentally and resuscitation was attempted.

1. Occulusion of trachea causes cardiac standstill. When obstruction is released within 13 minutes and artificial respiration with 100% oxygen is carried out, cardiac rhythm recovers by cardiac massage.

2. Occlusion of the main stem of left coronary artery causes ventricular fibrillation. It cannot be abolished by countershocks without release of obstruction. Ventricular fibrillation by occlusion of right coronary artery, and peripheral branch of left coronary artery can be both successfully removed by electric shocks.

3. Oversupply of acetyl choline causes cardiac standstill. This can be resuscitated satisfactorily by cardiac massage and injection of atropine.

4. Acute massive bleeding causes cardiac standstill. Once the cardiac rhythm stops, resuscitation is almost impossible. In such cases, ECG, or macroscopic observation of the heart rhythm do not serve as a danger signal of cardiac arrest. Adequate control of transfusion should be carried out with attention to the amount of lost blood, changes of blood pressure and pulsation.

5. Narcotics as barbiturates and chloroform cause cardiac arrest. As their prognoses are bad, prevention of their occurrences should be carried out before hand.

6. The effect of injection of epinephrine, procaine amide and cardiac massage upon the heart muscle were discussed. Especially, assisted massage should be carried out carefully, when the irritablity of heart muscle is increased.

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Experimental Studies on Cardiac Resuscitation

III. Studies on Disturbance of Cerebral Function Following Cardiac Arrest

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Cardiac resuscitation by way of restoration of cardiac rhythm has been described in my reports **I** & **II**, but the way of preventing cerebral damage following cardiac arrest has not yet been investigated. The reports on clinical cases of cardiac resuscitation claim that cases of eventual deaths in spite of restarted pulsation were due to irreversible cerebral damage. It is believed that the time for which the brain can stand anoxia is around 3 minutes based on the experiment on total occlusion of pulmonary artery by Weinberger *et al.*[1] As different time limits are reported due to different experimental approaches to this problem, in the case of discussing the time limit in cardiac arrest, cardiac arrest caused experimentally should be first considered. Such an experiment, however, has only been tried by Batelli in 1900.[2] Herein, the cerebral damage caused by ventricular fibrillation and obstruction of airway has been investigated by EEG and by histological examination of the brain tissue.

Experimental

The heart was exposed under positive pressure with 100% oxygen as described in Report **I**.

I. Ventricular Fibrillation and Brain Potentials

Method: In order to investigate the resistance of the brain to anoixa in different portions in the central nervous system, ventricular fibrillation has been induced electrically, and EEG was observed for 1 hour in 3 dogs and one cat.

Result: EEG findings.: (Fig. 1). EEG from the scalp or subcortex became rapidly flat following ventricular fibrillation. The potentials became small within 10–15 seconds which suggested loss of cerebral function. 1 minute later, EEG could hardly be observed. On the other hand, the amplitude of EEG from the subcortex became half

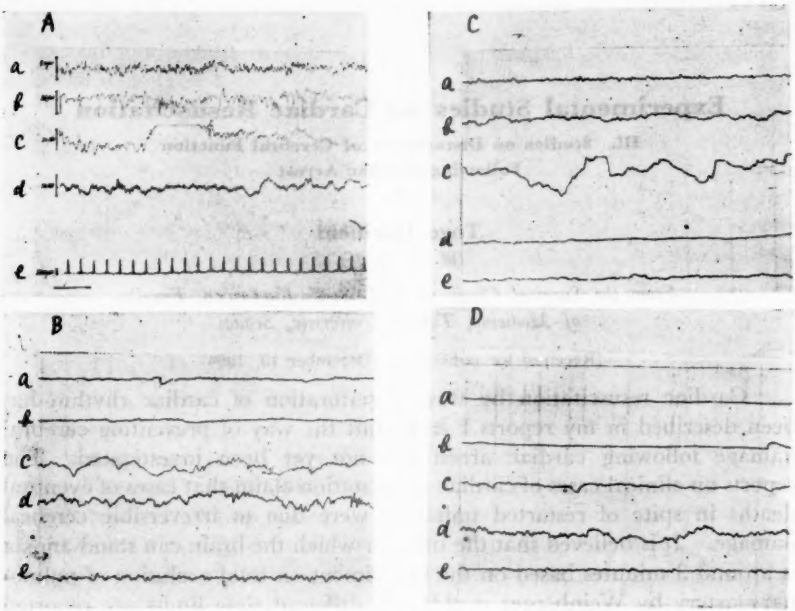


Fig. 1. Control: Brain wave and spinal wave during anoxia. dog. 13 kg.: A. Preoperative control: a. scalp, b. cortex, c. subcortex d. spinal cord. e. ECG; B. 10 minutes after V. F.: EEG from scalp and cortex flat; slow waves from subcortex and spinal cord. Function of spinal cord preserved fairly well; C. 20. minutes after V. F.: decrease of amplitude from subcortex and spinal cord. Damage of function evident; D. 40 minutes after V. F.: EEG from subcortex flat, increase of fast waves from the spinal cord.

of its size after 1 minute and continued to become smaller and disappeared about 30 minutes later, EEG from the spinal cord showed a tendency of decrease of amplitude and prolongation of frequency potential was still observed 60 minutes later. As EEG from the spinal cord showed smaller changes than that from the subcortex it is considered that the resistance of the spinal cord against anoxia is greater than the subcortex and its function is held longer. On the contrary, in cat, EEG from the spinal cord changed faster than that from the subcortex and almost disappeared 20 minutes later, while the potential from the subcortex still appeared 60 minutes later.

II. Ventricular Fibrillation lasting 2 and half Minutes

Method: After ventricular fibrillation lasted for 2 and 1/2 minutes, ventricular fibrillation was abolished electrically and the heart was re-

suscitated in 5 dogs.

Result: EEG findings: When ventricular fibrillation occurred, the amplitude decreased immediately and only small few microvolt waves remained for 15 seconds (Fig. 2, B); EEG completely disappeared about 1 minute later. When cardiac massage was started after ventricular fibrillation was left for 2 1/2 minutes beta waves (15 cycles per second (cps.), few microvolts) appeared with fluctuation of the base line, which however, disappeared 10 seconds later. 1 minute later, alpha waves appeared and the amplitude increased gradually. 4 minutes later, slow waves (30 microvolts) were superimposed on other slow waves (10 microvolts). Afterwards, remarkable changes were not seen and the amplitude did not recover to the preoperative level. After cardiac massage was done for 10–15 minutes, countershocks were applied to abolish ventricular fibrillation. The amplitude decreased slightly and alpha waves became lower than 10 microvolts. They, however, gradually recovered with appearance of slow waves (about 3 cps., 20–30 microvolts) (Fig. 2 C). After closure of the chest, the amplitude of brain waves decreased below the preoperative level. The wave pattern, however, recovered (Fig. 2 D). Postoperatively, the amplitude decreased and fast waves (30–40 cps., 20 microvolts) became predominant (Fig. 2., E, F & G). They may be regarded as a sign of damaged cerebral function. After the operation, corneal reflex and pupil reflex to light were both normal 24 hours later. The dogs, however, became restless, apathetic and forgot their habitual behaviours.

Histological findings: 2 months later, the dogs were sacrificed and their brains were extracted. The specimens were stained with hematoxylin and eosin or by Van Gieson-and Nissl's method. Macroscopically, no bleeding, necrosis, softening, etc., were observed. Examining the frontal lobe, the occipital lobe, the parietal lobe and the hippocampus of the cerebral cortex, vacuolization, atrophy, neuronophagia, shrinkage, etc. of the ganglion cells were seen throughout the field under the microscope. Scattered areas without ganglion cells were seen, and in many areas they were replaced by growth of glia cells. The so-called coagulation necrosis of the nerve cells which is said to appear during anoxia named after Spielmeyer was not observed. No abnormal findings of the nerve cells of Purkinje's cell and dental cells were seen in the cerebrum. Nerve cells of the subcortex, the brain stem, the pons, the medulla oblongata and the spinal cord were normal (Fig. 5).

III. Ventricular Fibrillation Followed by Cardiac Massage

Method: Cardiac massage was started soon after ventricular fibrillation left for 20 minutes. Then, ventricular fibrillation was abolished

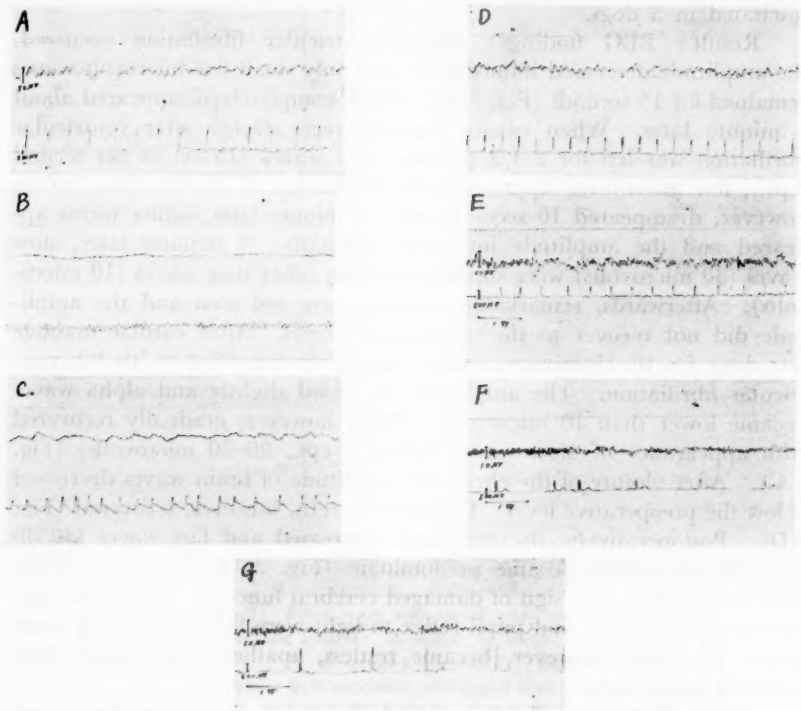


Fig. 2. EEG & ECG in case of defibrillated heart by countershock when massage was done for 14 minutes after V. F. lasted 2 minutes and a half beforehand. Dog. 16 kg.: A. preoperative tracing: EEG from scalp with ECG; B. 15 seconds after V. F.: EEG flat, ECG ventricular fibrillation; C. 14 min. after V. F.: V. F. removed by countershock, EEG slow waves & low voltage, ECG wide QRS due to bad electric intracardiac conduction; D. 26 min.: EEG recovered remarkably. ECG sinus rhythm T flat; E. 3rd day after operation: EEG fast waves, ECG low voltage; F. 11th day after operation: EEG same as the findings in 3rd day, ECG sinus arrhythmia; G. 34th day after operation: EEG fast waves decreased. ECG bradycardia.

electrically and resuscitation was attempted in 4 dogs.

Result: EEG findings; When cardiac massage was performed right after ventricular fibrillation, increase of amplitude and prolongation of frequency followed by gradual appearance of delta waves (Fig. 3 B) were observed. The EEG, however, never became completely flat. After ventricular fibrillation was removed followed by restoration of cardiac rhythm, decrease of amplitude, shortening of frequency, and appearance of fast

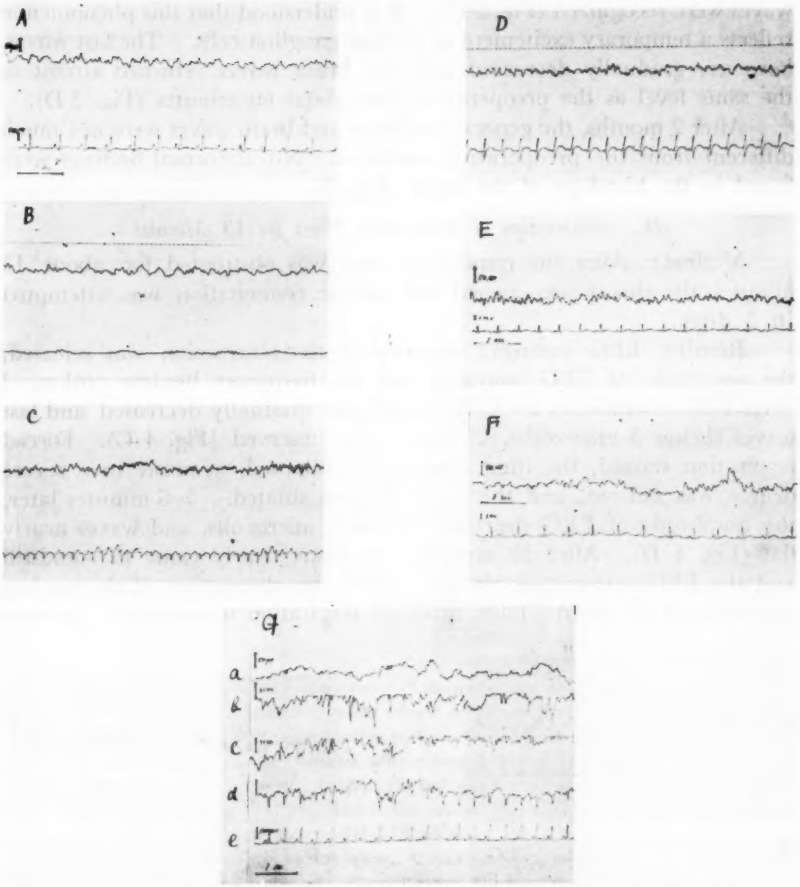


Fig. 3. EEG & ECG in case of defibrillated heart by countershock when cardiac massage was started soon after V. F. lasting for 32 minutes beforehand. Dog. 12 kg.: A. preoperative tracing; B. 20 minutes after V. F.: EEG slow waves, ECG ventricular fibrillation; C. countershock unsuccessful after 20 minutes. Continuous cardiac massage with injection of 100 mg. procaine amide into left ventricle. 32 minutes after V. F. Successful defibrillation by countershock. This graph is 1 minute after defibrillation, fast waves in EEG, ECG pararhythmia; D. 40 minutes: fast waves as in C with resemblance to preoperative tracing. ECG deep S, elevation of ST; E. 5th day: fast waves, ECG low voltage, sinus rhythm; F. 13th day: both EEG & ECG normal; G. 56th day: EEG from a. scalp, b. cortex, c. subcortex, d. spinal wave e. ECG. All normal. ECG superimposed on EEG & spinal wave.

waves were recognized (Fig. 3 C). It is understood that this phenomenon reflects a temporary excitement of cortical ganglion cells. The fast waves, however, gradually decreased and the brain waves returned almost to the same level as the preoperative after about 60 minutes (Fig. 3 D).

After 2 months, the general condition and brain waves were not much different from the preoperative condition. No abnormal findings were found in the histology of the brain (Fig. 6).

IV. Obstruction of Respiratory Tract for 13 *Minutes*

Method: After the respiratory tract was obstructed for about 13 minutes, the airway was opened and cardiac resuscitation was attempted in 5 dogs.

Result: EEG findings; As soon as the obstruction was released, the amplitude of EEG increased and the frequency became prolonged (Fig. 4 B). 3 minutes later, the amplitude gradually decreased and fast waves (below 5 microvolts, 20 cps.) were observed (Fig. 4 C). Forced respiration started, the lung completely collapsed, cyanosis in a severe degree was noticed, and the pupil became dilated. 5–6 minutes later, the amplitude of EEG decreased below 3 microvolts, and waves nearly flat (Fig. 4 D). After 10 minutes, the heart nearly came to standstill and the EEG was completely flat. After the obstruction of the trachea was released 13 minutes later, artificial respiration was started. As soon

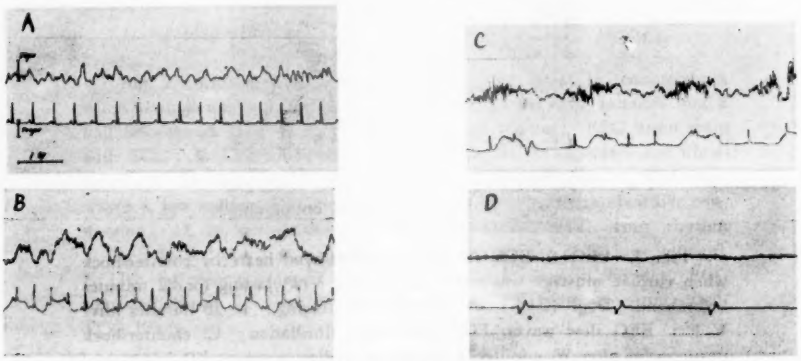
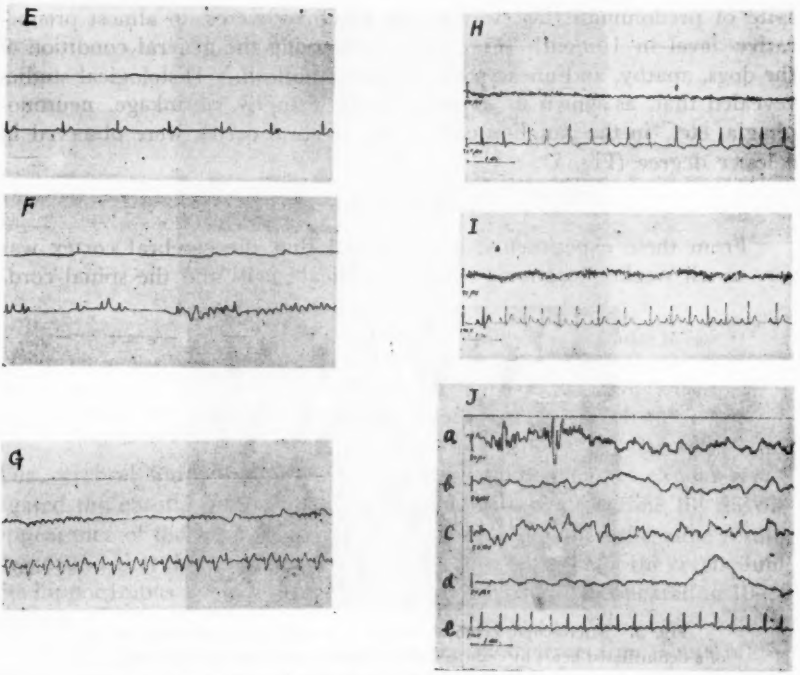


Fig. 4. EEG & ECG in the case of cardiac resuscitation when tracheal obstruction is relieved after 13 minutes. Dog 16 kg. : A. preoperative tracing ; B. 2 minutes after tracheal obstruction : slow waves superimposed on fast waves. exciting condition. remarkable respiratory fluctuation in EEG & ECG ; C. 3 minutes : fall of potentials in EEG. ECG ; bradycardia. rhythmical EMG appears due to frequent attack of convulsions ; D. 6 minutes : very low brain potentials, fast waves. disturbance of cerebral fuction evident. ECG ; automatie by lower center ; E. 10 minutes : brain



waves flat. ECG shows reappearance of sinus rhythm, bradycardia & weak heart beat; F. 13 minutes; after release of airway obstruction, artificial respiration with simultaneous massage performed transfer to V. F. 20 sec. after massage. EEG; G. 46 minutes after patent airway: increase of brain potentials. ECG; pararhythmia; H. 2nd day: fast waves, low voltage. EEG pararhythima; I. 17th day: fast waves, increase of potentials, improvement of cerebral function; J. 32nd day: EEG from a. scalp, b. cortex, c. subcortex, d. spinal wave, e. ECG both normal.

as cardiac massage was started, gradual appearance of EEG was observed, and signs of the recovery of cerebral function were noticed. In the fatal cases, in spite of appearance of cardiac rhythm, the brain potentials remained zero. After cardiac massage was performed for about 20 minutes, brain waves appeared with spontaneous respiration. Fast waves with very low voltage changed to slow waves with higher amplitude and approached to normal brain waves (Fig. 4 G). After closure of the chest, the amplitude decreased and showed a resembling preoperative brain wave. 2–3 weeks after operation, the amplitude decreased and fast waves appeared. It seemed at first that the cerebral function degenerated. It recovered gradually (Fig. 4 H & I) and in

spite of predominant fast waves, the EEG recovered to almost preoperative level in 1 month (Fig. 4 J). Observing the general condition of the dogs, apathy, and uneasy behavior were noticed. Histological studies revealed that, as shown in experiment *II*, atrophy, shrinkage, neuronophagia, etc., in the ganglion cells of the cerebral cortex were observed in a lesser degree (Fig. 7).

Discussion

From these experiments, it was found that the cerebral cortex was very much weaker to anoxia than subcortical cells and the spinal cord.

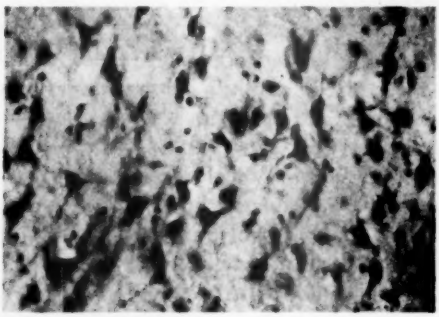


Fig. 5. Microscopic cerebral finding 2 months after operation in case of a defibrillated heart by countershock when massage was done for 14 minutes after V. F. lasted 2 minutes and a half beforehand: The 3rd layer of the cortex of the parietal lobe; Nissl's stain. Atrophy, vacuolization and neuronophagia of the ganglion cells observed. Shrinkage of dendrite remarkable.

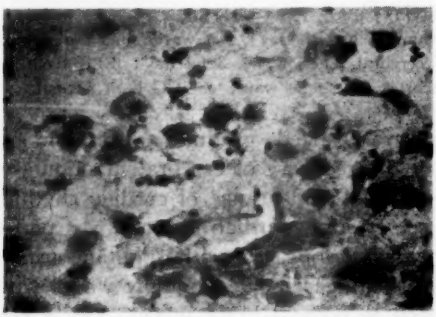


Fig. 6. Microscopic cerebral finding 2 months after operation in case of a defibrillated heart by countershock when cardiac massage was started soon after V. F. lasting for 32 minutes beforehand: The 3rd layer of the cortex of the frontal lobe; Nissl's stain. No shrinkage of the nerve cells. Nissl's granula seen.

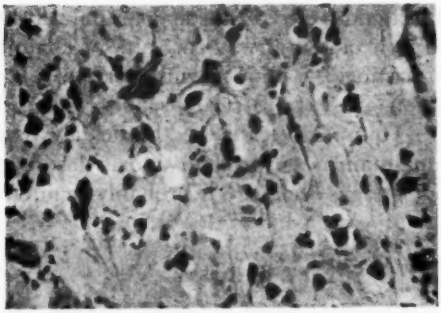


Fig. 7. Microscopic cerebral finding 1 month after operation in case of cardiac resuscitation when tracheal obstruction was relieved after 13 minutes: The 3rd layer of the cortex of the frontal lobe; Nissl's stain. Atrophy and neuronophagia seen in a slighter degree compared with the findings in Fig. 5.

The cerebral function stopped within about 15 seconds. Sugar *et al.*[3] ligated the carotid and vertebral arteries to measure the time till the disappearance of the brain potential occurred and obtained the same result; they found that the brain potential of the gray substance of the cerebellum, the hippocampus and the gray substance of cerebrum disappeared in 10–15 seconds.

Reviewing many articles regarding the recovering time limit of the brain from anoxia, quite different results have been reported. Such difference are due to the different experimental approach to the problem. Describing the studies in recent years, Kabat *et al.* in 1939[4] said the time limit to be 8 minutes by stopping the cerebral circulation, Weinberger *et al.* in 1940[1] said it to be 3 minutes and 10 seconds by obstructing pulmonary artery, Templeton *et al.* in 1949[5] said it to be 5 minutes by obstructing the superior and inferior vena cava simultaneously, and Kimoto *et al.* in 1954[6] claimed it to be 3 minutes by obstructing vena caval system and aorta. Batelli[2] said that the time limit to cause damage of central nervous system due to ventricular fibrillation was 15 minutes. The animals, however, all died within 24 hours, and it was obviously wrong that the time limit could be 15 minutes.

According to my experiment, the dogs showed abnormal behaviour after recovering from ventricular fibrillation lasting 2 minutes and a half. Abnormal brain waves were demonstrated 2 months later parallel with abnormal findings of the ganglion cells of cerebral cortex by histological study. These histological findings are completely compatible with those of Weinberger, Gildea, Morrison[7][8], etc. On the contrary, almost no abnormal findings were seen in those cases with immediate cardiac massage

after ventricular fibrillation, electroencephalographically and histologically. In cases of obstruction of the airway, in spite of complete suspension of oxygen to the lung lasting 3–8 minutes with disappearance of brain waves, the cerebral function are easy to recover and residual symptom of the cerebrum are few. This may be explained in such a way that weak cardiac contractions are maintained on gradual anoxia lasting for a long time. During this period, the gas exchange is probably maintained in the brain tissue.

Summary

Resuscitation of cardiac arrest was studied on the damage of cerebral function by EEG and histology.

1. The cerebral cortex has the least resisting ability to anoxia, and potentials of cerebral cortex disappear within 15 seconds after venticular fibrillation.

2. After ventricular fibrillation, the time limit to prevent irreversible damage of cerebral function is suspected to be 2 minutes.

3. If cardiac masage is started soon after ventricular fibrillation, no abnormal findings are recognized even when defibrillation is attempted after a long time.

4. In the case of cardiac arrest due to tracheal obstruction, it is possible to recover the heart without leaving permanent damage of cerebral function after release of the obstruction of the airway with restart of cardiac massage.

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Studies on a Method of Measuring Specific Gravity of Biological Fluid by Dilution

I. Measurement of Specific Gravity of Blood

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Acting on the consideration that, if we could easily and promptly measure the specific gravity of a liquid available only in a small quantity, with adequate precision and without requiring laboratory equipment, the practical value of such a method would be indeed high, not only in the field of medicinal study but also in scientific research in general, we devised the N-N (Nakazawa-Nakakai's) method of measuing the specific gravity of such a liquid in dilution, and based on this method, have contrived a N-N dilution hydrometer for practical use.

Hitherto, the method of measuring the specific gravity of liquids required either a specific-gravity bottle or an areometer, but the former is to be used in combination with a chemical balance and the latter is usable only when the tested liquid is available in a considerable quantity, so that both these appliances are not adapted to measure the specific gravity of a liquid of small quantity on the spot. In particular, the measurement of the spccific gravity of blood is rather difficult in comparison with many other liquids. Since Boyle[1] has ascertained in 1684 that blood is relatively much heavier than water, there have been devised many methods for measuring the specific gravity of blood, including the falling drop methods by Barbour & Hamilton[2] and Kagen[3], but these are seldom put to practical use in clinics, perhaps due to their complexity. In 1943, Phillips and his co-workers[4] perfected their copper sulfate method, and thereby the measurement of the specific gravity of blood came to be undertaken by clinical practitioners. In this method, however, there is the trouble of preparing many kinds of accurately diluted standard copper sulfate solutions, which are, moreover, limited in the times of repeated use.

In our N-N method such defects have been entirely eliminated, and it makes it possible to measure the specific gravity of a liquid in small amount, with ease, promptitude and precition.

If the specific gravity of blood, or that of whole blood and of serum, is measured, we can calculate the total serum protein, the hemoglobin concentration, the oxygen capacity of the blood as well as blood water etc. from the measured values, which would be of assistance in making diagnosis, deciding the principle of therapy and judging the prognosis and of great significance in a very wide scope in clinical practice as well as in medical study. Therefore, in this first report of ours on the subject, we will discuss the practical application of this method in measuring the specific gravity of blood, in collation with the copper sulfate method and the method of using Pulfrich's dipping refractometer.

Principle of N-N Dilution Method

In a N-N dilution hydrometer, a small ball of suitable specific gravity is sealed in a tube graduated to show at once the dilution and the specific gravity of a liquid tested in it. A fixed quantity of the tested liquid is then poured into the tube and carefully diluted with water or any other liquid of known specific gravity down to the precise concentration till the specific gravity ball stops to float or sink and shows no tendency to stir. Then the specific gravity of the tested liquid at the prevailing temperature may be read off the graduated scale indicating the level of the surface of the dilution. It may be needless to reiterate that when the tested liquid is specifically lighter than water, a liquid heavier than water and known specific gravity should be used to increase the total density by dilution.

N-N Dilution Hydrometer

Fig. 1 (A) shows a part of the lateral vertical section of the apparatus and Fig. 1 (B) a magnified partial vertical section of the specific gravity ball.

As shown in the Figs., the specification of this hydrometer consists in that a specific gravity ball room (6) is formed in a long graduated tube (1) with partitions (3) perforated or meshed so as a liquid will freely pass(4), between the bottom (2) of the tube and the level a little below the lowest point of the graduation (5) and a small hollowed hard-glass specific gravity ball (7) of a size not passing through the perforations or meshes of the partitions (about 1.5–2.5 mm. in diameter) is sealed in the room. The specific gravity ball room is readily washed by opening the stopper (8). The stopper (9) is used when a liquid is added to dilute the tested liquid.

One side of the transparent hydrometer tube is graduated beginning from 10 to 100 at every 0.5. This graduation is mainly for measuring the specific gravity of whole blood or serum, so that a reading of, say, 23.5 indicates that the specific gravity stands at 1.0235. On another side, the graduation begins at 2 and ends at 20, divided by every 0.1. This is for

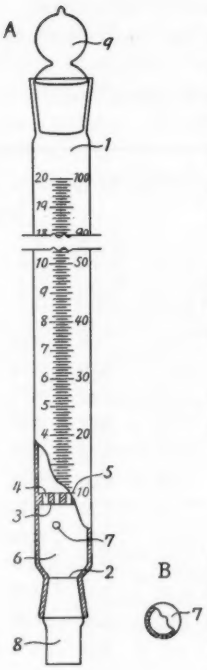


Fig. 1. N. N. Dilution Hydrometer.

use in testing cerebrospinal liquor, exudates, transudates, urine etc. and an indication of, say, 15.1 shows that the specific gravity of the tested liquid is 1.0151.

The capacities of the part of the tube below the lowest end of the graduation and above that end up to the mark of 20 on the scale for whole blood and serum are 2 cc. each.

When the tube is designed as above and the specific gravities of the specific gravity ball and the diluting liquid are of appropriate values calculated by means of the formula

Specific gravity × volume = mass

a reading of the scale may be made to indicate directly the specific gravity of the tested liquid.

Measurement of Specific Gravity of Whole Blood and Serum

When serum is to be tested, we have only to take a fixed volume of it with a graduated pipette into the hydrometer tube and to dilute it with water, but when it comes to whole blood which readily coagulates, another preliminary operation is called for. First, some water must be placed into the tube of the hydrometer (or test tube) and then the prescribed quantity of the blood must be taken up with a pipette immediately upon etting and poured into this water to cause hemolysis. Then water is added up to the required dilution.

Results of Measurement

I. When the specific gravity of the specific gravity ball is adjusted to 1.010 *to water of* 25°C

In the first series of my experiments, the specific gravity ball was made to have the specific gravity of 1.010 to water of 25°C, so that the graduation showed the specific gravity of whole blood and serum to water of 25°C, if 2 cc. of the tested liquids was used.

1) Measurement of whole blood and serum standardized at 25°C

Whole blood and serum from 13 normal cases, 16 hypertensives and 23 cases of various other diseases, 52 in total, were subjected to the test. A

part of the results is shown in Table I.

TABLE I

Measurement of Specific Gravity of Whole Blood and Serum standardized at 25°C (No. 3)

Case No.	Diseases	Specific gravity of serum (d_{25}^{25})		Specific gravity of serum (d_{25}^{25})		Serum protein %			Hemoglobin (g/dl) N.N. method
		N.N. dilution method	Copper sulfate method	N.N. dilution method	Copper sulfate method	N.N. dilution method (A)	Pulfrich's refracto-meter (B)	Difference (A~B)	
32	Disease of heart	1.049	1.049	1.028	1.028	7.83	7.69	0.14	10.4
33	"	1.047	1.046	1.025	1.025	6.67	6.79	0.12	10.4
34	"	1.049	1.048	1.024	1.024	6.28	6.42	0.14	11.7
35	Hemiplegia	1.058	1.058	1.028	1.028	7.83	8.02	0.19	14.4
36	Disease of kidney	1.047	1.047	1.025	1.024	6.67	6.55	0.12	10.5
37	"	1.056	1.056	1.023	1.023	5.90	6.08	0.18	15.2
38	Disease of liver	1.055	1.055	1.026	1.026	7.02	7.20	0.18	13.9
39	Asthma bronchiale	1.061	1.061	1.029	1.029	8.22	8.06	0.16	16.0
40	"	1.067	1.066 over	1.032	1.032	9.37	9.35	0.02	13.2
41	"	1.057	1.056	1.030	1.029	8.62	8.51	0.11	13.7
42	Tumor of lung	1.053	1.053	1.030	1.030	8.62	8.62	0.00	11.7
43	Abdominal tumor	1.052	1.052	1.029	1.028	8.22	8.21	0.01	11.5
44	Lues cerebri	1.055	1.055	1.030	1.030	8.62	8.71	0.09	12.7
45	Anaemia	1.048	1.048	1.030	1.030	8.62	8.71	0.09	9.1
46	"	1.045	1.045	1.029	1.029	8.22	8.10	0.12	7.9
47	Ankylostomiasis	1.057	1.056	1.029	1.029	8.22	8.21	0.01	14.0
48	Tetanus	1.053	1.053	1.0285	1.028	8.30	8.15	0.15	12.2
49	Neuropathy	1.060	1.060	1.030	1.030	8.62	8.49	0.13	15.2
50	"	1.061	1.061	1.031	1.031	9.00	8.92	0.08	15.4
51	"	1.054	1.054	1.028	1.028	7.83	7.89	0.06	12.8
52	"	1.062	1.061	1.0315	1.031	9.20	9.20	0.00	15.8

The values of specific gravity obtained by this method were in almost perfect agreement with those by copper sulfate method, the greatest divergence being under ± 0.001.

The total content of serum protein also approached that obtained with a Pulfrich's dipping refractometer, both the values completely coinciding in some cases. The largest difference here was the case where the refractometer showed 6.08% while our method registered a content of 5.90%. So, the maximum deviation of the values obtained by Pulfrich's dipping refractometer from these by our method was only 3%.

2) Method of correcting specific gravity to standard value at 25°C

The method to reduce the specific gravity obtained of whole blood and serum at a given temperature to the values at the standard temperature of 25°C, may be outlined as follows.

The formulae (1), (2) and (3) are deduced and the formula for the correction is obtained from them, as shown in Table II. Upon measuring the value of α_t, the formula for compensation for any temperature may be obtained.

TABLE II

Correction to the Standard Temperature 25°C

When S_t: Scale reading at t°C

S_{25}: Ditto at 25°C

α_t: Increase or decrease in volume according to change in temprerature of the diluted tested liquid of the specific gravity equal to that of the specific gravity ball

M_{25}: The mass of the tested liquid diluted to the specific gravity of the specific gravity ball at 25°C (the mass of 1 cc. of water at 25°C taken as equal to 1 g.)

V_{25}: Volume of M_{25}

V_t: Volume of tested liquid diluted to the specific gravity of the specific gravity ball at 25°C

β_t: Density of water at t°C

$$\frac{S_{25}}{V_{25}}=\frac{S_t}{V_t} \quad \cdots\cdots (1)$$

$$\frac{M_{25}}{V_{25}}=1.010 \quad \cdots\cdots (2)$$

$$\frac{M_{25}+\{V_t-V_{25}(1\pm\alpha_t)\}\frac{\beta_t}{0.99709}}{V_t}=1.010 \quad \cdots\cdots (3)$$

Formula for correction

$$S_t=S_{25}\left(1\pm\frac{\beta_t}{0.99709}\cdot\frac{\alpha_t}{\gamma}\right)$$

$$\gamma=1.010-\frac{\beta_t}{0.99709}$$

$$\text{in}\pm \begin{cases} +\text{means lower than 25°C} \\ -\text{means higher than 25°C} \end{cases}$$

$\therefore$ S_{25} is calculated out by multiplying with factors:

S_{10} :0.758	S_{15} :0.810	S_{20} :0.886	S_{26} :1.029
S_{11} :0.766	S_{16} :0.822	S_{21} :0.906	S_{27} :1.060
S_{12} :0.776	S_{17} :0.837	S_{22} :0.926	S_{28} :1.090
S_{13} :0.786	S_{18} :0.851	S_{23} :0.950	S_{29} :1.122
S_{14} :0.797	S_{19} :0.868	S_{24} :0.975	S_{30} :1.151

3) Measurement of α_t

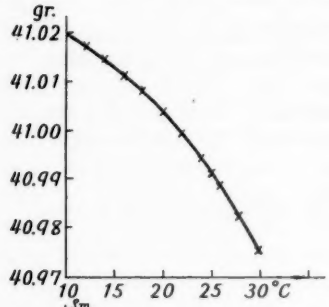
The tested liquid (diluted with water to the specific gravity of the specific gravity ball at 25°C or the relative specific gravity of 1.010 to water of 25°C), with which the specific gravity bottle of 10.265 cc. in capacity is filled overflows by expansion when heated. I weighed the solution in the bottle at every 2°C after wiping away the overflow, in the range of temperature of 10–30°C.

TABLE III

Measurement of α_t

Measured Values

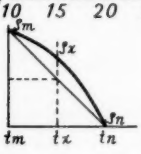
Temperature °C	10	12	14	16	18	20	22	24	25	26	28	30
Weights gr.	41.0192	41.0170	41.0145	41.0116	41.0082	41.0042	40.9996	40.9941	40.9912	40.9884	40.9826	40.9768



Values obtained from the Graph

Temperature °C	11	13	15	17	19
Weights gr.	41.0181	41.0158	41.0130	41.0099	41.0063

Temperature °C	21	23	27	29
Weights gr.	41.0020	40.9969	40.9855	40.9796



If $\rho_m \cdots \rho_n$ are the corrected values corresponding to temperatures $t_m \cdots t_n$, we get, by proportional equation,

the formula (1). $t_x = t_m + (\rho_m - \rho_x)\frac{(t_n - t_m)}{(\rho_m - \rho_n)}$ --------(1)

theoretically $t_x = f(\rho_x) = f(\rho_m + \overline{\rho_x - \rho_m})$ --------(2)

Error $\delta t = (1) \sim (2) \fallingdotseq f''(\rho_m)(\rho_m \sim \rho_n)^2/8 \fallingdotseq 10^{-8}$

The measured values indicated in Table III were obtained by this method. The measured values were plotted on a graph and the weights at the intermediate temperatures were read off the graph. These values are used for the calculation of α_t.

By the way, let us compute the error of the values thus obtained from the graph. If $\vartheta_m \cdots\cdots \vartheta_n$ are the corrected values corresponding to temperature $t_m \cdots\cdots t_n$, we get, by proportional equation, the formula (1). The error δ_t is represented by the difference of this formula (1) and the theoretically derived formula (2). The results of expanding the function and calculating the approximate value of its second derivative shows that the maximum deviation in this case is of the order of 10^{-8}. Therefore, the graph values may be deemed to be adequately correct.

4) Values of α_t

TABLE IV

Value of α_t and β_t

α_t: The mass of 1 cc. of water at 25°C taken as equal to 1 g.

t°C	α_t	β_t
10	0.0243×1/1.010×10.265	0.99973
11	0.0235× "	0.99963
12	0.0226× "	0.99952
13	0.0216× "	0.99940
14	0.0206× "	0.99927
15	0.0193× "	0.99913
16	0.0182× "	0.99897
17	0.0167× "	0.99880
18	0.0154× "	0.99862
19	0.0136× "	0.99843
20	0.0118× "	0.99823
21	0.0098× "	0.99802
22	0.0077× "	0.99780
23	0.0052× "	0.99756
24	0.0026× "	0.99732
26	0.0030× "	0.99681
27	0.0062× "	0.99654
28	0.0093× "	0.99626
29	0.0126× "	0.99597
30	0.0156× "	0.99567

The values of α_t calculated thus for various temperatures are shown in Table IV.

5) Measurement of specific gravity of serum of any given temperature

Using the formula for compensation from any temperature between 10—30°C to 25°C, obtained as given above, I proceeded with testing serum at various temperatures for their specific gravity (Table V). The samples used were 20 different aqueous dilutions of serum.

The temperature at the time of measurement (the temperature at which the tested liquid is diluted to the density so that the specific gravity ball stops all movement for floating or sinking; the expression is used in the same meaning hereunder) is measured, the indication of the hydrometer read, and reduced to the value at the standard 25°C as above, and the result expressed in the nearest four figures under decimal.

Example: In the Case 1 of Table V, the temperature at the time of measurement is 30°C and the reading of the hydrometer 26.5. Here,

TABLE V

Measurement of Specific Gravity of Serum at any Given Temperature

Case No.	N.N. dilution method				Pulfrich's refractometer	Difference (A~B)
	Temperature (°C)	Hydrometer reading	Specific gravity (d^{25}_{25})	Serum protein % (A)	Serum protein % (B)	
1	30	26.5	1.0305	8.80	8.71	0.09
2	21	30.5	1.0276	7.86	7.85	0.17
3	27	25.0	1.0265	7.26	7.38	0.12
4	29	23.0	1.0256	6.89	7.11	0.22
5	17	30.5	1.0255	6.86	6.68	0.18
6	19	27.5	1.0239	6.24	6.12	0.12
7	28	21.0	1.0229	5.85	5.79	0.06
8	14	28.0	1.0223	5.62	5.51	0.11
9	24	22.0	1.0215	5.30	5.47	0.17
10	10	28.0	1.0212	5.19	4.96	0.13
11	30	18.0	1.0207	5.00	5.14	0.14
12	15	24.5	1.0198	4.65	4.53	0.12
13	22	21.0	1.0194	4.46	4.56	0.10
14	15	23.5	1.0190	4.33	4.10	0.23
15	23	19.0	1.0181	4.00	4.01	0.01
16	13	22.5	1.0177	3.86	3.64	0.22
17	19	19.0	1.0165	3.36	3.24	0.12
18	16	19.0	1.0162	3.24	3.35	0.11
19	20	17.0	1.0155	3.00	3.02	0.02
20	14	19.5	1.0155	3.00	2.95	0.05

then $S_{30}=26.5$, and using this value in the formula $S_{25}=S_{30}\times 1.151$, I get $S_{25}=26.5\times 1.151=30.50$. The specific gravity at 25°C then may be given as 1.0305.

In these cases, the total contents of serum protein calculated from these value were very approximate to those obtained by use of Pulfrich's dipping refractometer, the maximum deviation being 3.86% per our hydrometer versus 3.64% per Pulfrich's dipping refractometer, amounting to 6.0% in ratio.

II. When the relative specific gravity of the specific gravity ball is adjusted to 1.002 *to water of* 15°C

If we could improve our hydrometer to make it applicable to measure, beside the specific gravity of whole blood and serum, also that of exudates, of transudates, of cerebrospinal liquor, of urine, etc., all with one hydro-

meter, it would be of wider utility. For realizing such an idea, we made a hydrometer of the same design, but with the specific gravity ball having a specific gravity of 1.002 to water of 15°C instead of 1.010 to water of 25°C. With this hydrometer, the required quantity of the tested whole blood and serum is reduced to 0.4 cc. per test.

For example, when the graduation of the hydrometer for testing blood is used, if the specific gravity ball stopped sinking or floating when the tested liquid was diluted from 0.4 cc. to just 6 cc., or to 1 :15 in volume, the tested liquid would have a difference of 15 times from water in specific gravity as that of the specific gravity ball of 1.002, that is, 1.030. Now, in this case, the mark reading 30 on our hydrometer shows that the volume of the dilution is 6 cc., and the graduation corresponds to the specific gravity of the tested liquid, so it is convenient to use 0.4 cc. of the tested liquid per test.

When using the graduation for measuring the specific gravity of exudates etc. on another side of the tube, the graduation is made similarly to correspond with the specific value of the tested liquid, when 2 cc. of the latter is taken for testing.

1) Method of correcting the result to standard value at 15°C

The method of correcting the results obtained from measurement of whole blood and serum (and of exudates, transudates, cerebrospinal liquor, urine etc.) at a given temperature to the standard values relative to water of 15°C is as follows.

From the equations (1), (2) and (3), the formula for the required compensation is deduced, as shown in Table VI, the values of α_t are got by measurement and thus the formula for correction for every degree of temperature is derived.

2) Measurement and values of α_t

Similar to the procedure as described in 3) and 4) of I above, the mass (Table VII) when the standard of temperature is taken at 15°C and the values of α_t (Table VIII) are obtained.

3) Method of correcting specific gravity of whole blood and serum from d_{15}^{15} to d_{25}^{25}

Since the specific gravity of whole blood and serum is usually expressed in the terms of the specific gravity of these liquids of 25°C to water of 25°C (d_{25}^{25}), the values obtained at 15°C of both the tested liquid and water (d_{15}^{15}) must be converted to (d_{25}^{25}).

The formula for the required conversion is obtained as shown in Table IX. In the case of serum, we may neglect the difference and usually assume $d_{15}^{15} \fallingdotseq d_{25}^{25}$.

4) Measurement of specific gravity of serum at a given temperature

The results are shown in Table X. For the samples to test, I used

TABLE VI

Correction to the Temperature 15°C

When S_t: Scale reading at t°C

S_{15}: Ditto at 15°C

α_t: Increase or decrease in volume according to change in temperature of the diluted tested liquid of the specific gravity equal to that of the specific gravity ball

M_{15}: The mass of the tested liquid diluted to the specific gravity of the specific gravity ball at 15°C (the mass of 1 cc. of water at 15°C taken as equal to 1 g.)

V_{15}: Volume of M_{15}

V_t: Volume of tested liquid diluted to the specific gravity of the speicfic gravity ball at 15°C

β_t: Density of water at t°C

$$\frac{S_{15}}{V_{15}}=\frac{S_t}{V_t} \quad \cdots\cdots (1)$$

$$\frac{M_{15}}{V_{15}}=1.002 \quad \cdots\cdots (2)$$

$$\frac{M_{15}+\{V_t-V_{15}(1\pm\alpha_t)\}\frac{\beta_t}{0.99913}}{V_t}=1.002 \quad \cdots\cdots (3)$$

Formula for correction

$$S_t=S_{15}\left(1\pm\frac{\beta_t}{0.99913}\cdot\frac{\alpha_t}{\gamma}\right)$$

$$\gamma=1.002-\frac{\beta_t}{0.99913}$$

in $\pm$ $+$ means lower than 15°C

$-$ means higher than 15°C

$\therefore$ S_{15} is calculated out by multiplying with factors:

S_{10}: 0.795	S_{16}: 1.080	S_{21}: 1.645	S_{26}: 2.261
S_{11}: 0.811	S_{17}: 1.170	S_{22}: 1.773	S_{27}: 2.421
S_{12}: 0.844	S_{18}: 1.282	S_{23}: 1.903	S_{28}: 2.583
S_{13}: 0.889	S_{19}: 1.415	S_{24}: 2.002	S_{29}: 2.689
S_{14}: 0.927	S_{20}: 1.524	S_{25}: 2.135	S_{30}: 2.978

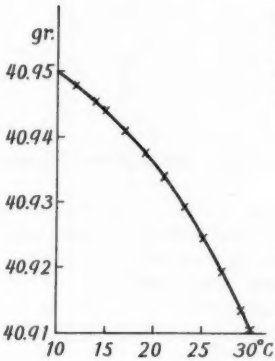
20 different dilutions at different temperatures, taking 0.4 cc. of them per test.

Measuring the temperature at the time of measurement and reading the indication on the dilution hydrometer, the values standardized at 15°C were calculated by the formula for compensation and the nearest values at four figures below the decimal was taken. These values represent d_{15}^{15}, but since serum only was tested, the values were assumed to fall under the generalization $d_{15}^{15}\fallingdotseq d_{25}^{25}$ above.

Example: In the Case 15 of Table X, the temperature at the time of measurement is 18°C and the hydrometer registered 14.0. Then, $S_{18}=14.0$. Substituting this value into $S_{15}=S_{18}\times 1.282$, we find $S_{15}=$

TABLE VII

Measurement of α_t



Measured Values

Temperature °C	10	12	14	15	17	19
Weights gr.	40.9494	40.9480	40.9456	40.9442	40.9412	40.9370

Temperature °C	21	23	25	27	29	30
Weights gr.	40.9331	40.9287	40.9243	40.9193	40.9136	40.9104

Values obtained from the Graph

Temperature °C	11	13	16	18	20	22
Weights gr.	40.9488	40.9469	40.9428	40.9392	40.9351	40.9309

Temperature °C	24	26	28			
Weights gr.	40.9267	40.9220	40.9164			

TABLE VIII

Values of α_t and β_t

α_t : The mass of 1 cc. of water at 15°C taken as equal to 1 g.

t°C	α_t	β_t
10	$0.0040 \times {}^1/1.002 \times 10.265$	0.99973
11	$0.0036 \times$ "	0.99963
12	$0.0031 \times$ "	0.99952
13	$0.0022 \times$ "	0.99940
14	$0.0012 \times$ "	0.99927
16	$0.0017 \times$ "	0.99897
17	$0.0035 \times$ "	0.99880
18	$0.0057 \times$ "	0.99862
19	$0.0082 \times$ "	0.99843
20	$0.0103 \times$ "	0.99823
21	$0.0126 \times$ "	0.99802
22	$0.0150 \times$ "	0.99780
23	$0.0175 \times$ "	0.99756
24	$0.0197 \times$ "	0.99732
25	$0.0223 \times$ "	0.99707
26	$0.0249 \times$ "	0.99681
27	$0.0278 \times$ "	0.99654
28	$0.0308 \times$ "	0.99626
29	$0.0336 \times$ "	9.99595
30	$0.0375 \times$ "	0.99567

TABLE IX

Correction of Specific Gravity d_{15}^{15} to Ditto d_{25}^{25} of Whole Blood and Serum

When the specific gravity of water of 25°C is taken as unit, that of water of 15°C is 1.00206 ..... (1)

When the mass of unit volume at 15°C: x g.

$x - d_{25}^{25} = M_d$ ..... (2)

$x = 1.00206\ d_{15}^{15}$ ..... (3)

Formula for Correction:

$d_{25}^{25} = 1.00206\ d_{15}^{15} - M_d$

Theoretically, d_{25}^{25} is calculated from the measured values of M_d of tested liquid at various specific gravities

Practically, the error in the results may be neglected in clinics, if the M_d values of whole blood and serum of persons in normal health is measured and calculation is made thereupon using the following formulae:

d_{25}^{25} of whole blood $= 1.00206 \times d_{15}^{15}$ of whole blood $- 0.0037$

d_{25}^{25} of serum $= 1.00206 \times d_{15}^{15}$ of serum $- 0.0021$

N. B.: In calculating the mass of the tested liquid, 1 cc. of water at 25°C is taken to weight 1 g.

TABLE X

Measurement of Specific Gravity of Serum at any given Temperature

Case No.	N.N. dilution method				Pulfrich's refractometer	Difference (A~B)
	Temperature (°C)	Hydrometer reading	specific gravity ($d_{15}^{15} \fallingdotseq d_{25}^{25}$)	Serum protein % (A)	Serum protein % (B)	
1	27	13.0	1.0315	9.20	8.82	0.38
2	23	15.5	1.0295	8.41	8.15	0.26
3	13	31.5	1.0280	7.82	7.74	0.08
4	21	16.5	1.0271	7.46	7.35	0.11
5	18	21.5	1.0276	7.68	7.20	0.48
6	24	12.5	1.0250	6.65	6.92	0.27
7	13	29.0	1.0258	6.96	6.88	0.08
8	18	18.0	1.0231	5.93	6.34	0.41
9	10	29.5	1.0235	6.08	6.23	0.15
10	19	16.0	1.0226	5.73	5.86	0.13
11	15	22.5	1.0225	5.69	5.40	0.29
12	15	21.0	1.0210	5.11	4.81	0.30
13	14	20.0	1.0185	4.15	4.51	0.36
14	16	18.0	1.0194	4.50	4.47	0.03
15	18	14.0	1.0179	3.92	4.16	0.24
16	16	17.0	1.0184	4.12	3.94	0.18
17	16	15.5	1.0167	3.44	3.66	0.22
18	14	18.0	1.0167	3.44	3.39	0.05
19	17	13.5	1.0158	3.12	3.06	0.06
20	11	19.0	1.0154	2.96	2.84	0 12

TABLE XI

Measurement of Specific Gravity of Whole Blood and and Serum at Given Temperature (No. 1)

Case No.	Diseases	Whole Blood					Serum				Serum protein %		
		N.N. dilution method				Copper sulfate method	N.N. dilution method			Copper sulfate method			
		Temperature (°C)	Hydrometer reading	Specific gravity (d^{15}_{15})	Specific gravity (d^{25}_{25})	Specific gravity (d^{25}_{25})	Temperature (°C)	Hydrometer reading	Specific gravity ($d^{15}_{15} \fallingdotseq d^{25}_{25}$)	Specific gravity (d^{25}_{25})	N.N. dilution method (A)	Pulfrich's refractometer (B)	Difference (A∼B)
1	Normal case	30	17.5	1.0521	1.0506	1.052	24	12.5	1.0250	1.025	6.65	6.80	0.18
2	"	22	33.0	1.0585	1.0570	1.058	20	18.0	1.0274	1.027	7.62	7.42	0.20
3	"	14	62.5	1.0579	1.0564	1.056	14	31.0	1.0287	1.028	8.10	7.89	0.21
4	"	15	60.0	1.0600	1.0585	1.059	18	22.0	1.0282	1.028	7.92	8.06	0.14
5	"	17	45.0	1.0527	1.0512	1.052	18	21.5	1.0276	1.026	7.68	7.02	0.66
6	"	15	61.0	1.0610	1.0595	1.060	18	23.0	1.0295	1.030	8.42	8.58	0.16
7	"	17	52.0	1.0608	1.0593	1.059	17	24.5	1.0287	1.028	8.10	8.21	0.11
8	"	13	68.5	1.0609	1.0594	1.059	15	31.0	1.0310	1.030	9.00	9.03	0.03
9	"	13	70.0	1.0608	1.0593	1.059	19	21.0	1.0297	1.029	8.49	8.21	0.28
10	"	13	70.0	1.0622	1.0607	1.060	17	23.5	1.0275	1.028	7.64	8.28	0.64
11	"	13	63.5	1.0565	1.0550	1.055	13	33.0	1.0293	1.028	8.34	8.32	0.02
12	"	13	60.5	1.0538	1.0523	1.053	15	30.0	1.0300	1.029	8.61	8.60	0.01
13	Diseases of digestive tract	28	21.5	1.0555	1.0540	1.055	21	15.0	1.0255	1.026	6.86	7.09	0.23
14	"	26	20.5	1.0464	1.0449	1.045	21	14.5	1.0239	1.024	7.24	6.30	0.06
15	"	13	48.5	1.0431	1.0415	1.042	16	24.5	1.0265	1.026	7.23	7.70	0.47
16	"	14	51.5	1.0477	1.0462	1.046	15	26.0	1.0260	1.025	7.03	7.20	0.17

17.94. The specific gravity of the serum is therefore 1.01794 or 1.0179.

The results obtained in these 20 cases, upon collation with the reading of Pulfrich's dipping refractometer in measuring the content of serum protein, show a maximum deviation in the case where the former stood at 4.15% and the latter at 4.51%, or a deviation of 8.1% in ratio.

5) Measurement of specific gravity of whole blood and serum at any given temperature

The results were obtained upon testing the whole blood and serum from 12 persons in normal health, 6 hypertensives, 9 pulmonary tuberculosis cases and 23 cases of other diseases, or 50 cases in total. 0.4 cc. each of whole blood and serum was used per test. A part of the results is shown in Table XI.

The values obtained from the dilution hydrometer readings at the temperature at the time of measurement are converted to the standard values at 15°C by the formula for correction. In the case of serum, these values d_{15}^{15} are given without further manipulation, but in the case of whole blood, the values d_{15}^{15} must be again corrected to get d_{25}^{25}.

Example: In the Case 10 of Table XI, in the case of whole blood, the temperature at the time of measurement stands at 13°C and the hydrometer registration at 70.0. Then, $S_{13}=70.0$, and using the correction formula, we get $S_{15}=62.23$. By the equation $S_{25}=1.00206\times d_{15}^{15}-0.0037$ we get $d_{25}^{25}=1.06068$. Thus, the specific gravity of the whole blood in this case is 1.0607.

In these 50 tests, the results were found to coincide with the results obtained by the copper sulfate method within the range of error of ± 0.0015.

The content of serum protein calculated from the results of our tests also showed values very nearly approximating those obtained by use of Pulfrich's dipping refractometer. The largest difference was seen in the case where the former registered 7.68% while the latter stood at 7.02%. The maximum deviation between the two sets of values is then 9.4% in ratio.

Note: For calculating the content of serum protein we used the nomogram (I) shown on page 83 of Yoshikawa's "Copper Sulfate Method".

Discussion and Conclusion

The author has conducted measurements of 102 samples of whole blood and serum each and 40 samples of serum with a newly designed N-N (Nakazawa-Nakai's) hydrometer and now I will summarize the main merits and demerits of this hydrometer recognized in analysing the outcomes of the tests, as a discussion of and a conclusion to the above, as follows.

The apparent points of merit are that

1) It is extremely simple in operation. Of course, no test room or laboratory equipment is needed. If only the tested liquid is available, the actual measurement of specific gravity takes only five minutes at the outside. It is practically useful.

2) If the specifications of the apparatus and the conditions of its use as the quantity of the tested liquid per test, the specific gravity of the specific gravity ball, the inner diameter and the length of the transparent meter tube, the fineness of the graduation of the scales, etc. are appropriately designed, the apparatus may be made adequate to measure the specific gravity of any liquid to any required degree of precision. Thus it may be of use in a laboratory too.

3) For measuring the specific gravity of whole blood, it has been usual to mix some anti-coagulant, but this addition is apt to occasion errors in the measured results. The N-N hydrometer does not make use of such an anti-coagulant.

As almost all fluids of the body, such as exudates, blood and cerebrospinal liquor, are soluable in water, water will do in any case for diluting the tested liquid, and no other reagents or any auxiliary means are required.

4) Since the required correction table for temperature has been drawn up, it may be used under any temperature on the spot.

5) Even in the case of a dark-colored liquid, e.g., whole blood, as it is diluted in the tube, there is no difficulty in discerning the movement or pause of the specific gravity ball.

6) For precision measurement of the liquid to be tested, the use of a good graduated pipette is of course recommended, but in the case where such an instrument is not available, the graduation on the tube is made to indicate the volume as well as the specific gravity, so that an approximate measurement is possible with this tube only.

7) The graduation given on the opposite side of the tube to that used for testing blood and serum makes it possible to measure the specific gravity of cerebrospinal liquor, of exudates, of transudates, of urine etc. to the unit of 10^{-4}. The areometer hitherto used for measuring cerebrospinal liquor, for example has a scale graduated in 4 degrees only between 1.001 and 1.010. On the results of my experiments with such non-blood fluids I will report in the near future.

As to the demerits of N-N hydrometer, I may point out that

1) Sometimes froth gathers on the surface of the liquid, so as to make accurate reading difficult. For assuring good mixture of the tested and the diluting liquids, the minor operation of slanting and reerecting the tube is repeated for a few times and bubbles are apt to form in the

liquid as it flows through the perforations of the partition plates during this operation. Therefore, frothing may be avoided by taking care to let the liquid flow gently through a part of the perforated plates. When froth has gathered, addition of a few drops of ether or lettering the tube stand for some time will effect disappearance of the froth. The scale may be read with clarity and precision thereafter.

2) The temperature of the tested liquid in the tube is apt to alter by the influence of the heat given off by the hand of the operator etc. A significant deviation of the temperature shown by the thermometer and the actual temperature of the liquid at the time of measurement will cause an error in the result. Such a deviation is caused by the difference of the temperature of the thermometer touching the liquid. For eliminating such an influence, the tube should be manipulated by holding parts distant from the liquid, such as the lower end of the stopper for cleaning. The thermometer should be left standing in the liquid for a few minutes to assure that the temperature of the two is equalized, and then the specific gravity ball should be reexamined for its movement. When the ball does not show any tendency to rise or sink in the liquid, then the temperature should be at once read and this value should be used as the temperature at the time of measurement. The use of a slender thermometer not longer than is practically necessary is recommended.

3) If the dilution has gone too far, the specific gravity ball will sink to the bottom and the measurement will fail. As the dilution further required may be surmised from the speed with which the specific gravity ball floats up, adequate care should be effective in preventing such a failure, but if once overdilution has been committed, we may accomplish the purpose by adding a known quantity of the tested liquid or cooling it by placing the tube in cold water.

4) On very rare occasions, bubbles will adhere to the specific gravity ball. Such bubbles scarcely cause inconvenience in practice, as they will float off the ball while the tested and the diluting liquids are mixed. If they do not come off then, a repetition of the mixing operation or a shake given to the tube will surely effect a release.

Note: When the temperature at the time of measurement stands at, say 16.5°C, for an utmost accuracy of results, the a_t for 16.5°C should be calculated and a formula for correction deduced from this value, but the use of the mean of the formulas $S_{15}=S_{16}\times 1.080$ and $S_{15}=S_{17}\times 1.170$, that is $S_{15}=S_{16.5}\times 1.125$ will hardly cause any error of consequence.

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Electrophoresis of Sera of Breast-fed Infants and Its Relation to Nutritional Dystrophy of Their Mothers

Studies on the Nutrition of Children in Hirosaki Area

(22nd Report)

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In the preceding papers[1)2)] I reported electrophoretic patterns of sera from children with or without nutritional dystrophy[3)4)5)] in Hirosaki area. In the present investigation electrophoretic analysis of sera from the patients of the age of from 1 to 24 months was carried out and the results obtained will be discussed from nutritional point of view.

Experimental

Method and Materials

Estimation of total protein and electrophoresis of sera were carried out in the same way as described in my previous paper[1)].

The infants subjected to this investigation were those who visited our Clinic and those who had been admitted into a nursing room. All the infants were divided into the following 6 groups (Groups I, II, III, IV, V and VI).

Group I included 32 cases of the patients who were of the age from 1 to 12 months and were exclusively fed on milk from the mothers without nutritional dystrophy.

Group II included 18 cases of the patients who were of the age from 1 to 12 months and were exclusively fed on milk from the mothers with nutritional dystrophy.

Group III included 25 cases of the patients who were of the age from 13 to 24 months and who were fed partly on an additional food (see below) and partly on milk from the mothers without nutritional dystrophy. The additional food, for infants of the age of 12 months or more, consisted chiefly of carbohydrate, that is to say, of large amount of rice-gruel and

small amount of vegetables and fishes. Meat and egg were scarecely taken by the inhabitants and also by the infants in this area.

Group IV included 6 cases of the patients of the age from 16 to 24 months, who were fed partly on the additional food and partly on milk from the mothers with nutritional dystrophy.

Group V included 12 cases of the infants who were of the age from 6 to 21 months and who were admitted into a nursing room on account of economical shortage of their family. They had been, therefore, undernourished at the time of their admission but they had been suffering from none of underlying diseases. Their body weight increased gradually during staying in the nursing room, but it still remained slightly lower than the normal at the time of this investigation.

Group VI included 8 cases of the patients of the age from 3 to 20 months, who had been fed on mother's milk for a few months of their life and thereafter fed chiefly on rice gruel. Clinical manifestations of nutritional dystrophy were frequently found in the mothers or/and the infants in this Group.

Results and Comment

The results obtained are given in Tables I, II, II, IV, V and VI, and are summarized in Table VII.

(1) Electrophoretic patterns of Groups I and II (Cf. Tables I, II and VII).

A decrease in albumin and an increase in alpha and gamma globulins were found in Group II with significant differences as compared with those in Group I. Judging from the normal values[6] of electrophoretic components of infants of the age from 3 to 12 months, it may be said that a decrease in albumin and an increase in gamma globulin were significant and alpha and beta globulins ranged near the upper limit of the normal values in Group II; and that, in Group I, electrophoretic patterns remained within the normal range, but showed a tendency to a decrease in albumin and an increase in alpha and beta globulin. The average of total protein is higher in Group II than in Group I, but no significant difference was found between the two groups.

(2) The electrophoretic patterns of Groups III and IV (Cf. Tables III, IV and VII).

The averages of albumin was lower in Group IV than in Group III, and those of total protein and of alpha, beta and gamma globulins were higher in Group IV than in Group III. The differences in these components were not significant between these two groups. As compared with the normal values[6] it may be said that a decrease in albumin and an increase in alpha (probably in beta and gamma) grobulins were found in

TABLE I

Electrophoretic Pattern of the Sera from the Breast-fed Infants whose Mothers are free from Clinical Manifestations of Nutritional Dystrophy

No. of case	Name, sex and age (months)	Clinical diagnosis	Total protein gm. per 100 cc.	Albumin %	Globulin (%)			A/G
					α	β	γ	
1	K.E. ♂ 1.	Acute pharyngitis	6.61	53.6	13.1	14.6	19.1	1.18
2	Y.Y. ♂ 1.	Eczema	5.21	64.6	12.0	13.5	9.9	1.82
3	G.Y. ♀ 1.	Acute pharyngitis	4.81	57.7	14.5	16.2	11.6	1.36
4	Y.H. ♀ 2.	Pertussis	5.91	69.6	10.6	10.6	9.2	2.29
5	K.T. ♂ 2.	Pertussis	5.15	58.1	15.3	17.1	9.5	1.39
6	I.K. ♂ 2.	Infantile beriberi	5.41	64.5	14.8	12.5	8.2	1.82
7	N.W. ♀ 3.	Acute bronchitis	5.85	62.2	14.6	13.5	9.7	1.65
8	F.M. ♀ 3.	Acute pharyngitis	5.31	53.4	20.2	17.4	9.0	1.15
9	Y.I. ♀ 3.	Congenital stridor	5.92	56.3	18.1	17.4	8.2	1.29
10	T.K. ♂ 4.	Angina	5.85	59.5	14.0	13.8	12.7	1.47
11	T.S. ♂ 4.	Varicella	5.38	59.4	14.6	16.1	9.9	1.46
12	N.K. ♂ 4.	Spastic hemiplegia	7.24	50.2	19.7	20.0	10.1	1.01
13	K.I. ♀ 5.	Patent ductus arteriosus	5.30	72.2	12.9	9.3	5.6	2.60
14	O.N. ♂ 6.	Acute pharyngitis	6.27	69.6	12.4	12.8	5.2	2.29
15	K.K. ♂ 6.	Acute pharyngitis	6.77	70.0	11.6	11.2	7.2	2.23
16	C.T. ♂ 6.	Acute pharyngitis	6.46	60.1	17.1	15.1	7.3	1.43
17	M.K. ♂ 6.	Acute pharyngitis	5.77	55.9	13.4	21.1	9.6	1.27
18	A.K. ♂ 7.	Acute pharyngitis	6.36	60.2	13.0	14.7	12.1	1.51
19	K.E. ♀ 7.	Acute pharyngitis	5.82	62.7	16.7	11.3	9.1	1.68
20	N.S. ♀ 7.	Acute pharyngitis	6.23	58.1	13.3	19.7	8.9	1.39
21	I.Y. ♀ 7.	Acute pharyngitis	5.87	56.2	15.9	14.6	13.3	1.28
22	S.K. ♀ 7.	Infantile diarrhea	5.71	58.6	16.0	13.3	12.1	1.42
23	K.M. ♀ 8.	Pertussis bronchitis	7.35	51.4	16.2	20.1	12.2	1.06
24	M.S. ♂ 9.	Acute bronchitis	6.41	48.2	18.7	16.6	16.5	0.93
25	Y.C. ♀ 9.	Neuropathy	6.55	51.3	14.4	16.9	17.4	1.05
26	N.K. ♀ 10.	Varicella	6.83	63.1	11.9	11.3	13.7	1.71
27	T.Y. ♀ 11.	Acute bronchitis	5.83	53.1	21.2	13.6	12.2	1.13
28	K.Y. ♀ 11.	Acute pharyngitis	6.23	56.7	19.4	13.8	10.1	1.31
29	I.A. ♀ 11.	Angina	6.33	49.5	17.9	17.6	15.0	0.98
30	K.K. ♀ 12.	Heine-Medin's disease	7.11	51.2	18.2	15.1	10.5	3.39
31	K.Y. ♀ 12.	Angina	5.35	53.6	17.2	17.6	11.6	1.16
32	K.T. ♂ 12.	Acute pharyngitis	6.71	61.3	13.2	15.6	9.9	1.59
		Average	6.05 (±0.23)	58.5 (±3.1)	15.3 (±0.7)	15.1 (±1.0)	10.9 (±1.1)	1.46 (±0.15)

TABLE II

Electrophoretic Pattern of the Sera from the Breast-fed Infants whose Mothers show Clinical Manifestations of Nutritional Dystrophy

No. of case	Name, sex and age (months)	Clinical diagnosis	Total protein gm. per 100 cc.	Albumin %	Globulin (%)			A/G
					α	β	γ	
1	S.Y. ♀ 1.	Acute pharyngitis	5.63	47.0	16.7	14.0	22.3	0.89
2	F.C. ♀ 3.	Bronchopneumonia	5.95	57.7	18.2	14.6	9.5	1.36
3	K.T. ♂ 4.	Subcutaneous abscess	6.77	54.2	15.5	18.3	12.0	1.18
4	I.T. ♂ 5.	Acute bronchitis	6.21	50.3	20.0	16.7	13.0	1.01
5	S.A. ♂ 5.	Acute laryngitis	6.33	51.3	18.4	18.0	12.3	1.05
6	K.I. ♀ 6.	Acute pharyngitis	6.41	62.4	13.7	13.4	10.5	1.66
7	I.E. ♀ 7.	Atrophy	7.30	52.0	17.3	16.5	14.2	1.08
8	K.H. ♂ 7.	Acute bronchitis	6.13	43.9	17.8	18.3	20.0	0.78
9	N.C. ♂ 7.	Premature, rickets	6.50	51.4	18.6	18.1	11.9	1.06
10	S.M. ♂ 8.	Acute pharyngitis, rickets	7.03	30.4	21.0	15.6	32.6	0.44
11	K.K. ♀ 9.	Pertussis	6.74	57.1	15.1	13.8	14.0	1.33
12	A.A. ♀ 9.	Varicella	6.01	50.9	16.0	16.6	16.5	1.02
13	K.K. ♂ 10.	Pertussis	7.03	46.8	17.7	17.8	17.7	0.88
14	J.H. ♀ 10.	Acute bronchitis	5.93	53.0	17.0	14.2	15.8	1.13
15	K.S. ♂ 8.	Acute pharyngitis	5.93	48.1	24.3	17.9	9.7	0.92
16	S.H. ♂ 12.	Acute pharyngitis	5.81	48.3	17.1	17.1	17.5	0.93
17	A.H. ♀ 12.	Epidemic vomiting	7.31	52.7	11.7	11.3	24.3	1.17
18	O.H. ♂ 12.	Acute bronchitis	6.21	51.4	18.5	16.0	14.1	1.06
		Average	6.40 (±0.26)	50.5 (±3.6)	17.4 (±0.1)	16.0 (±0.8)	15.9 (±2.9)	1.04 (±0.15)

both Groups III and IV, especially in the latter.

(3) Electrophoretic patterns of Groups V and VI (Cf. Tables V, VI and VII).

The infants of Group V and those of Group VI were of the body weight lower than the normal, but the former had been fed on milk formula and the latter had been fed on the diet, a large portion of which consisted of carbohydrate (rice-gruel).

As compared with the normal values[6] it may be said that beta globulin was increased in both Groups V and VI and that an increase in gamma globulin and a decrease in albumin and total protein were significant in Group VI, while considerable variations from the normal were not found in total protein, albumin and alpha and gamma globulins in Group V.

Yamada *et al.*[7] determined, by means of Cohn's method, the components of serum protein of the normal infants of the age under 24 months

TABLE III

Electrophoretic Pattern of the Sera from the Breast-fed Infants in Weaning Periode whose Mothers are free from Clinical Manifestations of Nutritional Dystrophy

No. of case	Name, sex and age (months)	Clinical diagnosis	Total protein gm. per 100 cc.	Albumin (%)	Globulin (%)			A/G
					α	β	γ	
1	T.A. ♀ 13.	Acute dyspepsia	6.62	59.4	13.8	13.8	13.0	1.46
2	S.R. ♀ 13.	Acute pharyngitis	6.53	46.9	15.6	15.6	21.9	0.88
3	O.T. ♂ 13.	Rickets	6.30	54.4	15.6	17.9	12.1	1.20
4	T.H. ♀ 13.	Acute pharyngitis	7.01	48.7	16.6	15.2	19.5	0.95
5	O.M. ♂ 13.	Acute bronchitis	6.91	59.0	16.3	16.1	8.3	1.44
6	F.E. ♀ 13.	Heine-Medins' disease	6.48	50.0	16.2	18.0	15.8	1.00
7	Y.S. ♀ 14.	Izumi fever	7.32	55.4	19.0	12.4	13.2	1.24
8	F.S. ♂ 15.	Acute laryngitis	5.50	47.0	16.8	15.6	20.6	0.89
9	S.N. ♂ 15.	Angina	6.18	56.7	14.4	11.5	17.4	1.31
10	I.M. ♀ 15.	Acute bronchitis	6.15	53.6	14.9	13.2	18.3	1.16
11	H.K. ♂ 15.	Acute pharyngitis	5.45	52.0	16.9	16.0	15.1	1.08
12	J.H. ♂ 16.	Acute bronchitis	6.35	51.1	20.9	14.2	13.8	1.04
13	M.Y. ♂ 16.	Hypochromic anemia	6.75	57.0	11.3	13.1	18.6	1.33
14	I.N. ♀ 16.	Acute bronchitis	5.68	51.0	18.4	16.3	14.3	1.04
15	H.Y. ♀ 17.	Angina	7.42	45.3	22.0	13.7	19.0	0.83
16	O.T. ♂ 17.	Measles	5.93	67.1	13.9	13.3	5.6	2.04
17	K.B. ♀ 17.	Idiocy	6.73	59.7	13.7	14.7	11.9	1.48
18	K.M. ♂ 17.	Angina	6.71	51.1	16.0	15.2	17.7	1.04
19	S.K. ♂ 18.	Acute bronchitis	5.91	52.1	15.3	12.6	19.9	1.09
20	A.Y. ♀ 18.	Pertussis	1.85	57.1	11.6	17.5	13.6	1.33
21	A.M. ♀ 18.	Thrush	6.73	43.8	22.6	16.8	16.8	0.78
22	S.S. ♂ 20.	Exanthema subitum	6.68	51.8	24.1	14.6	9.5	1.07
23	N.T. ♂ 23.	Acute pharyngitis	6.77	57.8	15.2	13.9	13.1	1.37
24	K.G. ♂ 23.	Parotitis epid.	6.78	53.3	15.7	14.5	17.5	1.14
25	S.A. ♂ 24.	Acute pharyngitis	6.57	59.5	12.7	11.4	16.4	1.47
		Average	6.49 (±0.18)	53.6 (±2.1)	16.3 (±1.2)	14.7 (±0.7)	15.3 (±1.5)	1.18 (±0.10)

and they reported that total protein, albumin, alpha, beta and gamma globulins were 6.30 g. per 100 cc., 62.6%, 13.7%, 12.0% and 13.8% respectively. These values for the normal infants in Japan showed a fairly good accordance with those reported by Karte[6], who investigated German normal infants by means of electrophoresis.

Yamada *et al.*[7] reported also that total protein, albumin, alpha, beta and gamma globulins were 6.16 g. per 100 cc., 45.7%, 21.0%, 20.5% and

TABLE IV

Electrophoretic Pattern of the Sera from the Breast-fed Infants in Weaning Periode whose Mothers showed Clinical Manifestations of Nutritional Dystrophy

No. of case	Name, sex and age (months)	Clinical diagnosis	Total protein gm. per 100 cc.	Albumin (%)	Globulin (%)			A/G
					α	β	γ	
1	J.S. ♂ 16.	Izumi fever	6.75	41.9	23.2	19.6	15.3	0.72
2	S.T. ♀ 17.	Angina	7.42	45.3	22.0	13.7	19.0	0.83
3	U.T. ♂ 19.	Bronchopneumonia	7.07	51.0	17.7	16.0	15.3	1.04
4	M.S. ♂ 21.	Acute pharyngitis, rickets	6.43	55.6	13.6	15.1	15.7	1.25
5	S.K. ♂ 22.	Heine-Medin's disease	7.08	47.2	21.3	16.3	15.2	0.89
6	H.T. ♂ 24.	Acute bronchitis	7.01	49.5	17.3	15.5	17.7	0.90
		Average	6.96 (±0.39)	48.4 (±4.8)	19.2 (±3.6)	16.0 (±1.9)	16.4 (±1.6)	0.94 (±0.18)

TABLE V

Electrophoretic Pattern of the Sera from the Infants Staying in a Nursing Room

No. of case	Name, sex and age (months)	N.D.* (Infant)	Total protein gm. per 100 cc.	Albumin (%)	Globulin (%)			A/G
					α	β	γ	
1	A.J. ♀ 6.	—	5.81	50.2	18.0	17.1	14.7	1.01
2	K.E. ♀ 7.	—	6.11	56.6	17.9	17.5	8.0	1.30
3	I.Z. ♂ 7.	—	5.81	56.3	16.3	15.6	11.8	1.27
4	N.S. ♀ 8.	—	6.11	55.7	14.6	16.6	13.1	1.26
5	K.M. ♂ 12.	—	7.11	53.9	12.3	14.8	19.0	1.17
6	M.S. ♂ 13.	—	6.61	54.1	15.5	13.8	16.6	1.18
7	I.T. ♂ 15.	—	6.21	56.0	11.9	14.8	17.3	1.27
8	S.J. ♂ 9.	—	6.53	52.1	14.9	15.6	17.4	1.09
9	A.T. ♀ 21.	—	6.43	55.4	13.0	14.7	16.9	1.24
10	Y.Y. ♂ 6.	—	6.13	56.0	13.2	11.7	19.1	1.27
11	K.Y. ♀ 10.	—	6.23	57.8	12.3	15.9	14.0	1.37
12	K.O. ♂ 12.	—	6.63	56.0	14.8	16.9	12.3	1.27
		Average	6.28 (±0.24)	55.3 (±1.4)	14.5 (±1.1)	15.3 (±0.8)	14.8 (±2.0)	1.23 (±0.12)

* N. D.=Nutritional Dystrophy.

12.8% respectively as the averages obtained from the infants of the age from 3 to 24 months who were of the body weight below 80% of their

TABLE VI

Electrophoretic Pattern of the Sera from the Infants with Starch Injury

No. of case	Name, sex and age (months)	Complications	N.D.*		Total protein gm. per 100 cc.	Albumin (%)	Globulin (%)			A/G
			Infants	Mothers			α	β	γ	
1	Y.A. ♂ 3.	Acute bronchitis	+	−	3.72	46.4	14.5	10.0	29.0	0.87
2	M.T. ♂ 4.	Starch injury	+	+	6.52	51.6	11.5	13.5	12.5	1.06
3	O.K. ♂ 7.	Starch injury, Stomatitis	+	−	5.37	58.8	15.8	12.4	13.4	1.40
4	M.M. ♂ 16.	Acute pharyngitis, Acute dyspepsia	+	+	6.68	54.6	17.6	11.3	16.6	1.20
5	S.H. ♀ 19.	Starch injury	−	−	6.23	46.8	18.3	17.0	17.9	0.88
6	O.S. ♀ 19.	Acute pharyngitis	−	+	6.51	42.5	17.7	15.3	24.5	0.77
7	M.M. ♀ 19.	Rickets	−	+	6.72	54.3	14.8	13.3	17.6	1.19
8	J.T. ♀ 20.	Bronchopneumonia	+	−	5.28	30.4	12.4	26.3	30.9	0.44
		Average			5.92 (±0.83)	48.1 (±7.0)	15.3 (±6.9)	14.8 (±4.4)	20.3 (±5.5)	0.97 (±0.25)

standard and who were not complicated by any obvious diseases.

The majority of the infants subjected to my own investigation were complicated with acute or chronic infections. According to Karte[6], acute and chronic infections cause profound alterations in electrophoretic patterns of sera of infants. Karte[6] reported that acute infections such as bronchopneumonia, purulent meningitis, purulent otitis, pyuria, peritonitis and certain cases of tuberculosis caused a decrease in albumin and an increase in alpha and gamma globulins and that an increase in alpha globulin was more conspicuous than that in gamma globulin; and that subacute or chronic infections caused a decrease in albumin and an increase in alpha and gamma globulins and the increase in gamma globulin was more marked than in alpha globulin; further that these patterns were found in the cases with congenital syphilis, primary infection of tuberculosis, atrophy and dystrophy with recurrent infections. In the cases with atrophy, according to Karte[6], a slight increase in gamma globulin (15–26%), a decrease in albumin and normal or slightly deminished total protein were found and the increase in gamma globulin was not ascribed to infections but to protein deficiency.

As already reported[1] a decrease in albumin accompanied by an increase in alpha, beta and gamma globulins was found in electrophoretic patterns of children who visited our Clinic as patients and who showed

TABLE VII

Total Protein and Electrophoretic Components of Groups I, II, III, IV, V and VI and Values for 'Normal Infants'

Group	Method of nutrition	Age of infants	N.D.* of mothers	Total protein gm. per 100 cc.	Albumin (%)	Globulin (%)		
						Alpha	Beta	Gamma
I	M.M.**	1-12 m.	(−)	6.05±0.23	15.3±3.1	15.3±0.7	15.1±1.0	10.9±1.1
II	M.M.**	1-12 m.	(+)	6.40±0.26	50.5±3.6	17.4±0.1	16.0±0.8	15.9±2.9
III	M.M. + rice gruel	13-24 m.	(−)	6.49±0.18	53.6±2.1	16.3±1.2	14.7±0.7	15.3±1.5
IV	M.M. + rice gruel	13-24 m.	(+)	6.96±0.26	48.4±4.8	19.2±3.6	16.0±1.9	16.4±1.6
V	Chiefly evaporated milk	6-21 m.		6.28±0.24	55.3±1.4	14.5±1.1	15.3±0.8	14.8±2.0
VI	Chiefly rice gruel	3-20 m.	(+)	5.29±0.83	48.1±7.0	15.3±6.9	14.8±4.4	20.3±5.5
	'Normal'	2-10 m.		6.34 (5.5-7.45)	64.1 (59.5-69.5)	13.6 (9.7-17.4)	11.3 (7.8-16.1)	11.0 (7.8-13.8)
		3-7 y.		6.94 (5.93-7.63)	62.6 (56.1-69.4)	10.7 (4.9-13.7)	11.1 (8.0-15.7)	15.6 (10.6-19)

** M.M. = Mother's milk.

clinical manifestations of nutritional dystrophy.

The similar patterns of electrophoresis as above mentioned—a decrease in albumin accompanied by an increase in alpha, beta and gamma globulins—were found[2] also in apparently healthy children except that they showed clinical manifestations of nutritional dystrophy.

Sato and I[9] found that a decrease in albumin accompanied by an increase in gamma globulin was in a remarkable degree found in the sick children who showed degeneration of hepatic cells with portal fibrosis in the biopsied livers.

Sato[9] reported that " vaquolige " degeneration of hepatic cells, fatty infiltration and portal fibrosis were frequently observed in the biopsied liver of children who showed clear symptoms of nutritional dystrophy and who were not suffering from any other severe illness at the time of biopsy.

In the present investigation, clinical manifestations of the mothers of those infants were taken up as an indicator of nutritional environment of their infants, because angular stomatitis, cheilosis, swelling of lingual papillae, hyperkeratosis and hyperpigmentation of the skin were not commonly seen among the infants who were fed on milk from the mothers with nutritional dystrophy (Cf. Kagaya[12]).

This finding—a rare occurrence of manifestations of nutritional dystrophy in breast-fed infants of the mothers with manifestations of nutritional dystrophy—will be explained without difficulty, if clinical symptoms of nutritional dystrophy in those breast-fed infants will have become clinically manifest only then, when they weaned (—they actually wean mostly beyond one full year—) and have begun to take the diet qualitatively peculiar to that district or to take food similar to that taken by their mothers with nutritional dystrophy.

Out of 50 cases of exclusively breast-fed infants, none showed clinical manifestations of nutritional dystrophy, whether or not their mothers were of nutritional dystrophy.

Out of 31 cases of the infants of the age of 12 months or more, 3 cases showed angular stomatitis, cheilosis and red swollen tongue.

It may therefore be suspected that a decrease in albumin accompanied by an increase in gamma globulin in electrophoretic patterns of the infants of Group IV and VI is related to probable hepatic changes arisen from deficient nutrition.

How to explain the decrease in albumin accompanied by an increase in gamma globulin in the exclusively breast-fed infants of Group II?

Arakawa *et al.*[10] found that enlargement of the liver was frequently observed among the infants who were fed exclusively on milk from mothers with nutritional dystrophy, and they suspected that this hepatic enlargement was related to probable changes in ingredients of mother's milk.

Morinaga[11] of our Laboratory found that methionine content was lower in milks from mothers with nutritional dystrophy than in those from healthy mothers.

It may therefore be assumed such a possibility that a decrease in albumin accompanied by an increase in gamma globulin in Group II is ascribed to 'hepatic changes' which are caused by alterations in ingredients of milk from mothers with nutritional dystrophy.

Summary

1. Mothers in Hirosaki area show, as has been repeatedly shown by us, clinical symptoms of nutritional dystrophy.

2. Exclusively breast-fed infants of these mothers, as has been shown by Kagaya, do not show clinical signs of nutritional dystrophy.

3. These infants, however, show in electrophoretic pattern of serum protein, a decrease in albumin and an increase in gamma globulin.

4. So these breast-fed infants without clinical symptoms of nutritional dystrophy show a close nutritional connection with their mothers with distinct clinical symptoms of it in that peculiar electrophoretic pattern of serum protein.

Conclusions

Infants exclusively fed on milk of the mothers with clinical symptoms of nutritional dystrophy generally do not show manifest symptoms of nutritional dystrophy. But these apparently healthy infants do show a serum picture of nutritional dystrophy—an increase in gamma globulin with a decrease in albumin, as is shown in electrophoretic pattern of serum protein of children with nutritional dystrophy.

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Hepatic Enlargement of Children with Nutritional Dystrophy

Studies on the Nutrition of Children in Hirosaki Area

(23rd Report)

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Sato and Arakawa[1] reported 3 cases of hepatomegalic liver in children with chronic miliary tuberculosis and suggested that a main cause of hepatic enlargement must be sought rather in the nutritional deficiency[2)3)] prevalent among children in this area than in their tuberculous infection itself.

In the present paper an incidence in hepatic enlargement and its relation to nutritional dystrophy among children in this area will be reported.

Experimental

Materials and Method

The palpation of the liver was made on the right nipple line and measured in centimeters below the costal margin. (The liver palpable 4 cm., for example, means that the liver was palpable on the right nipple line as long as 4 cm. below the costal margin).

The children subjected to this investigation were those who were attending primary schools, of which one is situated at Hirosaki city and the other two at rural villages (Funazawa village and Miyoshi village).

Results

Hirosaki city (Cf. Table I).

Boys :—The cases with nutritional dystrophy were found in 62 (14.4%) out of 428 cases. The cases with palpable liver and those with the liver palpable 4 cm. or more were found in 16% and in 3.2% of boys with

TABLE I

Incidence of Palpable Liver and Its Relation to Clinical Manifestation of Nutritional Dystrophy of Children in Hirosaki City

Sex	Age years	Total number of the cases	Clinical manifestation of nutritional dystrophy (+) Liver: Palpable	(+) Liver: Not palpable		(−) Liver: Palpable	(−) Liver: Not palpable	
Boy	6-7	58	2	(1)*	10	3	(2)*	43
	7-8	64	3	(1)	5	6	(1)	50
	8-9	120	1	(0)	17	11	(0)	91
	9-10	92	2	(0)	11	11	(0)	68
	10-11	94	2	(0)	9	9	(0)	74
		428	10	(2)	52	40	(3)	326
			(16.1%)	(3.2%)	(93.9%)	(10.9%)	(0.7%)	(89.1%)
			62 (14.4%)			366 (85.6%)		
Girl	6-7	69	0	(0)	7	4	(1)	58
	7-8	102	0	(0)	7	4	(0)	89
	8-9	119	0	(0)	4	4	(0)	111
	9-10	85	0	(0)	2	5	(0)	78
	10-11	35	0	(0)	0	0	(0)	35
		410	0	(0)	20	19	(1)	371
			(0%)	(0%)	(100%)	(4.8%)	(0.26%)	(95.2%)
			20 (4.8%)			390 (95.2%)		

( )* represents number of cases with the liver palpable 4 cm. or more.

nutritional dystrophy respectively, and in only 10.9% and in only 0.7% of boys without nutritional dystrophy.

Girls :—The cases with nutritional dystrophy were found in 20 (4.8%) out of 410 cases. The cases with palpable liver and those with the liver palpable 4 cm. or more found in none of girls with nutritional dystrophy, and in 4.8% and 0.26% of girls without nutritional dystrophy respectively.

Funazawa village (Cf. Table II).

Boys :—The cases with nutritional dystrophy were found in 151 (47.6%) out of 317 cases. The cases with palpable liver and those with the liver palpable 4 cm. or more were found in 30.4% and 8.6% of boys with nutritional dystrophy respectively, and in only 12.0% and in only 4.2% in those without nutritional dystrophy.

Girls :—The cases with nutritional dystrophy were found in 82 (26.2%) out of 312 cases. The cases with palpable liver and those with the liver palpable 4 cm. or more were found in 8.5% and in 1.2% of girls with nutritional dystrophy respectively, and in 8.2% and 0.8% of girls without

TABLE II

Incidence of Palpable Liver and Its Relation to Clinical Manifestation of Nutritional Dystrophy of Children in Funazawa Village

Sex	Age years	Total number of the cases	Clinical manifestation of nutritional dystrophy (+) Liver: Palpable	(+) Liver: Not palpable		(−) Liver: Palpable	(−) Liver: Not palpable	
Boy	6-7	42	15	(2)*	12	2	(0)*	13
	7-8	46	5	(2)	14	0	(0)	27
	8-9	65	4	(0)	25	4	(0)	32
	9-10	62	5	(5)	23	9	(6)	21
	10-11	47	7	(3)	13	5	(1)	22
	11-12	56	7	(1)	18	0	(0)	31
		317	46	(13)	105	20	(7)	146
			(30.4%)	(8.6%)	(69.6%)	(12.0%)	(4.2%)	(88.0%)
			151 (47.6%)			166 (52.4%)		
Girl	6-7	37	3	(0)	9	3	(0)	22
	7-8	48	1	(0)	9	4	(0)	34
	8-9	57	1	(0)	16	3	(0)	37
	9-10	55	1	(0)	10	4	(1)	40
	10-11	49	0	(0)	9	3	(1)	37
	11-12	66	1	(1)	22	2	(0)	41
		312	7	(1)	75	19	(2)	211
			(8.5%)	(1.2%)	(91.5%)	(8.2%)	(0.8%)	(91.8%)
			82 (26.2%)			230 (73.8%)		

nutritional dystrophy.

Miyoshi village (Cf. Table III).

Boys:—The cases with nutritional dystrophy were found in 131 (79.9%) out of 164 cases. The cases with palpable liver and those with the liver palpable 4 cm. or more were found in 35.1% and in 12.9% of boys with nutritional dystrophy respectively, and in only 27.2% and in only 9.0% of boys without nutritional dystrophy.

Girls:—The cases with nutritional dystrophy were found in 100 (67.5%) out of 148 cases. The cases with palpable liver and those with the liver palpable 4 cm. or more were found in 31.0% and in 10.0% of girls with nutritional dystrophy respectively, and in 20.8% and in 10.4% of those without nutritional dystrophy.

COMMENT

As will be seen from Table IV in which the results obtained were summarized, the higher the incidence in nutritional dystrophy, the higher

TABLE III

Incidence of Palpable Liver and Its Relation to Clinical Manifestation of Nutritional Dystrophy of Children in Miyoshi Village

Sex	Age years	Total number of the cases	Clinical manifestation of nutritional dystrophy (+) Liver Palpable	(+) Liver Not palpable		(−) Liver Palpable	(−) Liver Not palpable	
Boy	6-7	19	4	(2)*	8	3	(0)*	4
	7-8	31	10	(3)	16	1	(1)	4
	8-9	35	11	(4)	19	1	(1)	4
	9-10	39	12	(4)	19	4	(1)	4
	10-11	40	9	(4)	23	0	(0)	8
		164	46	(17)	85	9	(3)	24
			(35.1%)	(12.9%)	(64.9%)	(27.2%)	(9.0%)	(72.8%)
			131 (79.9%)			33 (20.1%)		
Girl	6-7	31	3	(0)	7	1	(1)	20
	7-8	29	9	(4)	12	1	(1)	7
	8-9	36	6	(2)	22	3	(2)	5
	9-10	36	9	(4)	22	3	(0)	2
	10-11	16	4	(0)	6	2	(1)	4
		148	31	(10)	69	10	(5)	38
			(31.0%)	(10%)	(69.0%)	(20.8%)	(10.4%)	(79.2%)
			100 (63.5%)			48 (32.5%)		

TABLE IV

Incidence of Palpable Liver and Its Relation to Nutritional Dystrophy of Children

Sex	Regions	Total number of cases	Number of cases with nutritional dystrophy	Number of cases with palpable liver	Number of cases with the liver palpable 4 cm. or more
Boy	Hirosaki city	428	62 (14.4%)	50 (11.6%)	5 (1.1%)
	Funazawa village	317	151 (47.6%)	66 (20.8%)	20 (6.3%)
	Miyoshi village	164	131 (79.9%)	55 (33.5%)	20 (12.1%)
Girl	Hirosaki city	410	20 (4.8%)	19 (4.6%)	1 (0.2%)
	Funazowa village	312	82 (26.2%)	26 (8.3%)	3 (0.9%)
	Miyoshi villlage	148	100 (67.5%)	41 (27.7%)	15 (10.1%)

was the incidence in the cases with palpable liver or in those with the liver palpable 4 cm. or more. This tendency was observed in boys as well as in girls, though clinical manifestations of nutritional dystrophy were less frequently observed in girls than in boys.

As to the pathogenesis of the liver enlgargement, Sato[4] basing upon biopsy findings of the enlarged liver of children with nutritional dystrophy, concluded that the enlargement of the liver would be ascribed rather to hydropic degeneration of hepatic cells than to fatty degeneration of these cells and that these pathological changes in hepatic cells were caused by high-carbohydrate-low-protein diet[4] which has been and is prevalent among the inhabitants in Hirosaki area.

Summary and Conclusions

Enlarged liver was more frequently found in children with nutritional dystrophy than in those withuot it.

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Hepatic Enlargement of Breast-fed Infants and its Relation to Nutritional Dystrophy of their Mothers

Studies on the Nutrition of Children in Hirosaki Area

(24th Report)

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According to Arakawa *et al.*[1] the incidence of hepatic enlargement was higher among the children with nutritional dystrophy than among those without it.

In the present paper, hepatic enlargement of the breast-fed infants and its relation to nutritional dystrophy of their mothers and to the riboflavine content in the mother's milk will be reported.

Experimental

Materials and Method

The materials subjected to this investigation were restricted to those who were exclusively breast-fed, of the age under 12 months and were free from any complaints of illness.

These infants were selected from those who attended at a Baby show which was held on August 28, 1935 in two rural villages (Miyoshi-village and Rokugo-village, both situated at Tsugaru District in Aomori Prefecture).

These infants were divided into two groups: Group A consisted of those who were fed on the milk from the mothers without nutritional dystrophy and Group B consisted of those who were fed on the milk from the mothers with nutritional dystrophy.

Riboflavine content in mother's milk was estimated by means of lumiflavin-fluorometric method[2].

The palpation of the liver was made on the right nipple line and measured in centimeters below the costal margin. The "liver palpable 3 cm.", for example, means "the liver was palpable and 3 cm. long below

the costal margin on the nipple line ".

Results and Comment

Relation between nutritional dystrophy of mothers and hepatic enlargement of the breast-fed infants (Cf. Tables I and II).

TABLE I

Relation between Enlargement of the Liver of Breast-Fed-Infants and Riboflavine Content in Milk from the Mothers with or without Nutritional Dystrophy

Group	No. of case	Age (m.) and sex	Liver (cm.)	Riboflavine in mother's milk (γ%)
Group A (Mothers without nutritional dystrophy)	1	2. ♂	3	34.3
	2	2. ♂	2	35.5
	3	2. ♀	1.5	37.2
	4	2. ♂	—	46.3
	5	2. ♀	1.5	26.9
	6	3. ♀	—	22.3
	7	3. ♂	0.5	26.8
	8	4. ♂	2.0	32.4
	9	4. ♀	—	49.4
	10	4. ♀	—	20.3
	11	4. ♀	—	22.3
	12	4. ♀	—	22.3
	13	4. ♀	4.0	48.0
	14	4. ♀	1.0	33.4
	15	5. ♀	—	36.7
	16	5. ♀	—	26.6
	17	5. ♂	—	38.6
	18	5. ♂	3.5	36.0
	19	5. ♂	2.5	33.4
	20	5. ♀	—	31.5
	21	7. ♀	—	35.1
	22	7. ♀	—	35.3
	23	7. ♀	1.5	40.3
	24	11. ♂	2.0	20.6
	25	11. ♀	—	25.0
	26	12. ♀	1.5	30.0
			Average	32.7±2.7
Group B (Mothers with nutritional dystrophy)	27	6. ♀	1.8	28.3
	28	2. ♀	2.5	39.9
	29	2. ♀	1.8	37.2
	30	2. ♀	3.5	19.2
	31	5. ♀	3.5	25.6
	32	4. ♀	1.8	20.8
	33	4. ♂	1.0	18.9
	34	7. ♂	1.8	30.0
	35	6. ♂	3.5	23.3
	36	7. ♀	1.0	15.3
	37	4. ♂	1.8	30.2
	38	5. ♂	—	19.4
	39	6. ♂	1.0	16.5
	40	1. ♀	1.8	30.9
	41	3. ♂	1.8	25.9
	42	4. ♂	1.0	14.8
	43	3. ♂	1.8	24.2
	44	4. ♀	3.5	23.2
	45	6. ♂	3.5	31.6
	46	6. ♀	3.5	29.2
	47	4. ♀	2.5	32.4
	48	1. ♂	2.5	16.3
	49	4. ♂	3.5	11.5
	50	7. ♀	1.0	28.5
			Average	24.6±3.3

Out of the 50 cases examined, 14 cases were those without palpable liver, and of which 13 cases (92.8%) belonged to Group A and only one

TABLE II

Relation between Nutritional Dystrophy of Mothers and Enlargement of the Liver of the Breast-fed Infants

Group	Nutritional dystrophy of the mothers	The liver of the breast-fed infants (cm. below the costal margin on the right nipple line)			
		Not palpable (cases)	Less than 3 cm. (cases)	3 cm. or more (cases)	Total
A	(−)	13 (50.0%)	10	3 (11.5%)	26
B	(+)	1 (4.1%)	16	7 (29.1%)	24
	Total (cases)	14	26	10	50

cases to Group B. On the other hand, the cases with the liver palpable 3 cm. or more were 10 cases out of the 50 cases examined, and of which 7 cases (70.0%) belonged to Group B and only 3 cases to Group A.

In the normal infants of the age under 12 months, the liver was palpable 1 to 2 fingers breadth[3] or 2 to 3 cm.[4][5] below the costal margin on the right nipple line.

Concerning Japanese healthy infants, Matumoto and Tsukine[6] reported that the liver was not palpable in 303 (41.1%) out of 734 cases of the infants of the age under 6 months and in 569 (53.5%) out of 1064 cases of the infants of the age from 7 to 12 months, and they also reported that the liver palpable 3 cm. or more was found in 166 (9.2%) out of 1798 cases of the infants of the age under 12 months.

In the present investigation, the liver was not palpable in 13 (50.5%) out of the 26 cases of Group A, and in only one case (4.1%) out of the 24 cases of Group B. On the other hand, the liver which was palpable 3 cm. or more was found in 7 cases 29.1%) of Group B and 3 cases (11.5%) of Group A.

It may therefore be concluded that the incidence of the cases in which the liver was palpable 3 cm. or more was higher in Group B than in Group A and that the incidence of the cases without palpable liver was lower in Group B than in Group A.

Relation between the hepatic enlargement of the breast-fed infants and riboflavine content of their mother's milk (Cf. Table III).

According to Kagaya[7], one of us, there was a close relation between ariboflavinotic sigins (such as angular stomatitis, cheilosis and atrophy or/and swelling of the lingual papillae) and the blood riboflavine level of the children with nutritional dystrophy.

As will be seen from Table I, the averages of ribofiavine content in

TABLE III

Relation between Riboflavine Content of Mother's Milk and Enlargement of the Liver of Breast-fed Infants

Riboflavine content in mother's milk (γ%)	The liver of the breast-fed infants (cm. below the costal margin on the right nipple line)			
	Not palpable (cases)	Less than 3 cm. (cases)	3 cm. or more (cases)	Total
Below 25.0	5	8	4	17
21.5~35.0	2	13	4	19
Above 35.1	7	5	2	14
Total (cases)	14	26	10	50

mother's milk were $32.7\pm2.7\,\gamma\%$ and $24.6\pm3.3\,\gamma\%$ in Group A and Group B respectively, that is to say, riboflavine content was significantly lower in the milk from the mothers with nutritional dystrophy than in that from those without nutritional dystrophy. But, as will be seen from Table III, any significant relations were not found between the riboflavine content in mother's milk and the incidence of the cases with or without liver enlargement.

According to Sebrell *et al.*[8)9)10)] gross fatty liver was said to develop in riboflavine deficiency.

We are of the opinion that ariboflavinotic signs, which were found commonly among children in Hirosaki area, are not to be taken merely as symptoms of ariboflavinosis, but outgh to be considered as one of manifestations of nutritional dystrophy[11)12)].

Arakawa *et al.*[1)] found that the incidence of the cases in which the liver was palpable 4 cm. or more was higher in children with nutritional dystrophy than in those without it. Sato and Arakawa[13)] reported 3 cases of hepatomegalic liver in chronic miliary tuberculosis of children and described that a main cause of hepatic enlargement must be sought in the nutritional dystrophy prevalent among children in this area.

The components of mother's milk were decidedly altered by thiamine deficiency of the lactating mothers[14)].

Agostini[15)] reported, after milk analysis of many pellagrous mothers, that the milk showed a composition not different from that of the normal in fats and sugars, but that it showed a deficiency in casein and excess in salts.

Lucaletto[15)] called atention to the deficiency of iron in the milk of pellagrins.

Simonini[15] found that, in addition to an increase in salt and a decrease in casein, there was also a deficiency in iron, and he also suspected the presence of unidentified toxic principles in the milk of pellagrins.

From the present investigation, it may therefore be concluded that the high incidence of hepatic enlargement of the infants who were fed on the milk from the mothers with nutritional dystrophy, is not to be ascribed merely to the low riboflavine content in the milk, but to possible alteration in other factor or factors in the milk of the mothers with nutritional dystrophy.

Summary and Conclusions

The high incidence of hepatic enlargement was found among the infants who were fed on the milk from the mothers with nutritional dystrophy.

The hepatic enlargement of the breast-fed infants seemed to be closely related to nutritional dystrophy of the lactating mothers, but it may be not merely ascribed to the low riboflavine content in the milk of those mothers.

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Liver Biopsy Findings in Children with Nutritional Dystrophy

Studies on the Nutrition of Children in Hirosaki Area

(25th Report)

By

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A high incidence of hepatic enlargement among children with nutritional dystrophy[1)2)3)4)] in the Hirosaki area was reported by Arakawa *et al.*[5)6)] from our Department.

In the present paper I shall report biopsy findings of the liver of children with nutritional dystrophy.

Experimental

Materials and Method

The needle biopsy was performed with a Mitsuki needle[7)] and the subcostal approach was employed on the mid-clavicular line in all the cases. The biopsied specimens were fixed with formalin and embedded in carbowax. Fat was stained with Sudan III, and glycogen with Best's carmine.

The children subjected to this investigation were those who showed marked manifestations of nutritional dystrophy[3)] (angular stomatitis, cheilosis, swelling and/or atrophy of lingual papillae, hyperpigmentation of the skin, and palpable or enlarged liver) and who were not being complicated with other severe illnesses other than nutritional dystrophy itself.

Plant cell-like appearance of the hepatic cells—this term was used by Gillman and Gillman[8)]—shows such histological features that the hepatic cell is markedly swollen and its cytoplasm contains little stainable materials so that it appears rarefied, while the membrane of hepatic cells is sharply stained. The vacuolated, watery or plant cell-like appearance,—all these words were used by Gillman and Gillman[8)],—seems to represent the same histological features as those described as hydropic or " vacuolige "

degeneration[9][10] or protein depletion[11].

Results

The results obtained are given in Table I (A, B).

The liver was palpable below the costal margin on the right nipple line in all the cases, of which 20 cases were with the liver palpable 3 cm. or more.

TABLE I (A)

Liver Biopsy Findings of Children with Nutritional Dystrophy

No. of case (No. of biopsy)	Name, sex and age (y.m.)	Liver palpable cm.	Complications
1 (269)	S.M. ♂ 0.4	4	Rickets+Starch injury
2 (275)	T.O. ♂ 0.6	2	Starch injury
3 (49)	S.M. ♂ 1.2	3	
4 (218)	T.S. ♂ 1.3	2	Acute pharyngitis
5 (146)	H.H. ♀ 1.3	3	Pertussis
6 (45)	T.O. ♂ 1.4	4.5	
7 (261)	N.S. ♀ 1.10	1.5	Acute bronchitis
8 (271)	K.H. ♂ 2.0	1.5	Diarrhea
9 (232)	K.Y. ♂ 2.3	3.5	Rickets
10 (212)	H.Y. ♂ 7.5	3	Acute pharyngitis
11 (189)	H.S. ♀ 2.5	1.5	
12 (21)	H.O. ♂ 2.8	3	
13 (15)	M.K. ♂ 2.8	5	
14 (264)	Y.T. ♂ 3.0	1.5	
15 (223)	M.M. ♂ 3.0	3	
16 (236)	T.I. ♂ 3.2	4	Rickets
17 (177)	T.I. ♂ 3.2	1.5	
18 (156)	K.N. ♂ 3.10	3	Acute bronchitis
19 (226)	S.A. ♂ 4.0	2	Lymphadenitis colli tuberculosa
20 (184)	Y.K. ♂ 4.3	1.5	
21 (241)	K.H. ♀ 4.7	3	Ascariasis
22 (287)	T.T. ♂ 4.9	5	Oxyuriasis
23 (164)	M.K. ♂ 4.9	3	
24 (165)	T.I. ♂ 5.0	3	
25 (138)	A.K. ♀ 5.0	3	
26 (241)	M.K. ♂ 5.2	3	Izumi fever
27 (225)	Y.Y. ♂ 5.10	2	Stomatitis aphthosa
28 (127)	T.K. ♂ 6.1	7	Stomatitis aphthosa
29 (163)	Y.H. ♂ 6.3	3	Acute pharynigits
30 (262)	K.N. ♀ 7.3	4.5	Izumi fever

TABLE I (B)

Liver Biopsy Findings of Children with Nutritional Dystrophy

No. of case (No. of biopsy)	Histological findings								
					Fatty				
	Cloudy swelling	Plant cell like appearance	Basophilia	Glycogen	droplets	drops	Necrosis	Diffuse fibrosis	Other findings
1 (269)	−	−	−		+	+	−	−	
2 (275)	−	−	−		+	++	+	−	
3 (49)	++	+	−		−	−	−	++	Thickning of arterial wall
4 (218)	−	+++	−	+	−	−	+	−	
5 (146)	−	+	−		−	−	−	−	
6 (45)	++	−	−		−	−	−	+	
7 (261)	+	++	+		−	−	+	−	
8 (271)	−	++	+	++	−	−	+	+	
9 (232)	−	+++	−		−	−	−	−	Eosinophilic coagulum in Disse's space
10 (212)	−	+++	−	+++	−	−	−	−	
11 (189)	−	++	+		−	−	−	−	
12 (21)	+	−	−		−	−	−	+	
13 (15)	+	+	−		−	−	−	++	
14 (264)	−	±	+		−	−	−	−	
15 (223)	−	+++	−	+++	−	−	−	++	Eosinophilic coagulum in Disse's space and eosinophilic infiltration in portal tracts
16 (236)	−	+++	−	+++	−	−	−	+	
17 (177)	−	±	+		−	−	−	−	
18 (156)	−	++	−		+	−	−	−	
19 (226)	−	+++	−	+++	+	−	−	±	
20 (184)	−	+++	+		−	−	−	−	
21 (241)	−	++	−		−	−	+	++	
22 (287)	−	++	−		−	−	−	+	Eosinophilic infiltration in portal tracts
23 (164)	+	−	+		−	−	−	−	
24 (165)	−	++	+		−	−	−	−	
25 (138)	+	−	−		−	−	−	−	
26 (241)	−	++	+	+++	−	−	−	−	
27 (225)	−	+++	−	+++	−	−	−	+++	
28 (127)	+	+	−		−	−	−	++	
29 (163)	+	−	+		+	+	−	−	
30 (262)	+	−	++		−	−	−	−	

Histological findings of the biopsied livers

Cloudy swelling was found in 10 cases (Cases 3, 6, 7, 12, 13, 23, 25, 28, 29 and 30).

Plant cell-like appearance of the hepatic cells was found in 22 cases (Cases 3, 4, 5, 7, 8, 9, 10, 11, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 24, 26, 27 and 28). There was plenty of glycogen, but no neutral fat was demonstrated in the hepatic cells which showed plant cell-like appearance in hematxylin-eosin stain (Cf. Figs. 1, 2 and 3).

Fatty droplets or drops in hepatic cells were found in 5 cases (Cases 1, 2, 18, 19 and 29), of which 4 cases were under the age of 4 years (Cf. Fig. 4).

Basophilic stain of the hepatic parenchyma was found in small areas in lobules, where the cellular boundary was not distinct. These basophilic areas, to a variable extent, were found in 11 cases (Cases 7, 8, 11, 14, 17, 20, 23, 24, 26, 29 and 30), in which cloudy swelling or plant cell-like appearance of hepatic cells was also observed in other portions of the same lobules.

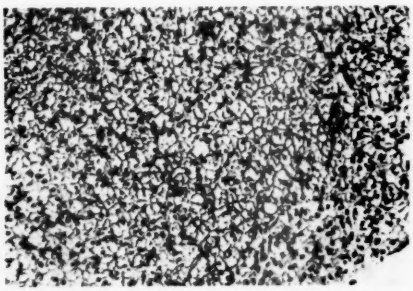

Fig. 1. Plant cell-like appearance of hepatic cells (liver biopsy); Hematoxylin eosin stain, Case 4.

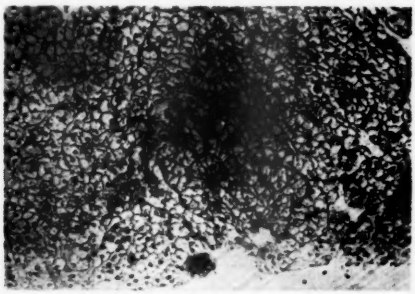

Fig. 2. Plant cell-like appearance of hepatic cells (liver biopsy); Hematoxylin eosin stain, Case 27.

Zonal necrosis was found in 5 cases (Cases 2, 4, 7, 8 and 21), all of which were under the age of 2 years (Cf. Fig. 5).

Diffuse fibrosis was found in 12 cases (Cases 3, 6, 8, 12, 13, 15, 16, 19, 21, 22, 27 and 28), and infiltration of eosinophils in the portal tracts was observed in 2 cases (Cases 15 and 22) (Cf. Fig. 6).

Eosinophilic coagulum in the space of Disse was found in 2 cases

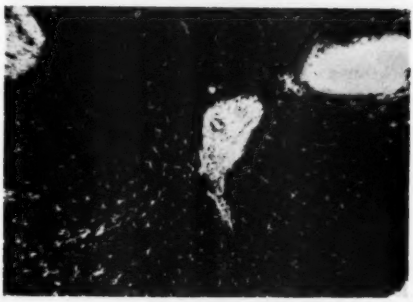

Fig. 3. Glycogen in hepatic cells (liver biopsy) ; Best's stain, Case 10.

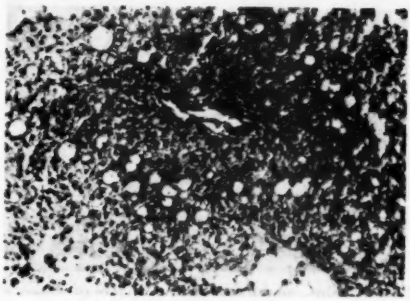

Fig. 4. Fatty infiltration of the liver (liver biopsy) ; Hematoxylin eosin stain, Case 2.

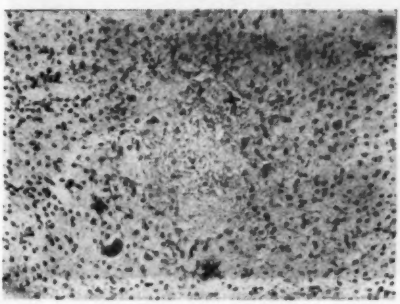

Fig. 5. Zonal necrosis (liver biopsy) ; Hematoxylin eosin stain, Case 7.

(Cases 9 and 15) (Cf. Fig. 7).

Thickning of the arterial wall was found in Case 3 (Cf. Fig. 8).

Comments

According to Gillman and Gillman[8], fatty liver was found in 54 out

Fig. 6. Portal fibrosis (liver biopsy) ; Hematoxylin eosin stain, Case 15.

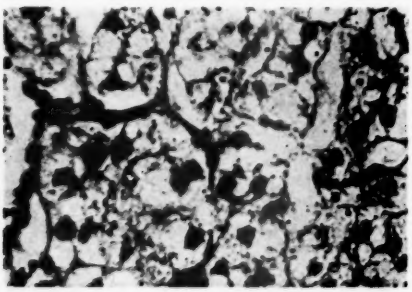

Fig. 7. Eosinophilic coagulum in the space of Disse (liver biopsy) ; Hematoxylin eosin stain, Case 15.

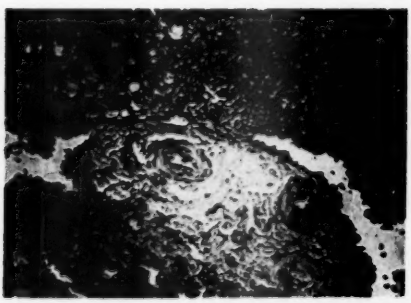

Fig. 8. Thickning of arterial walls (liver biopsy) ; Hematoxylin eosin stain, Case 3.

of 217 of the biopsied livers from African pellagrins, of which 39 were under the age of 10 years (especially under 3 years), while plant cell-like appearance of the hepatic cells was a common finding in the biopsied livers of adult pellagrins. The higher incidence of fatty liver among children than among adults, was ascribed partly to disturbed metabolism, which might be associated with an underdeveloped pancreas, and the persistence of the fetal portion of the adrenal cortex.

Plant cell-like appearance of the hepatic cells was, according to Gillman and Gillman[8], characteristic of " anoxic liver " and this histological finding was to be indistinguished from that described by Elman anß Heifetz in dogs on a low protein diet.

Gillman and Gillman[8], therefore, concluded that the plant cell-like appearance of hepatic cells of African pellagrins was to be ascribable to a depressed process of oxidation due to high-carbohydrate-low-protein diet.

Gõmez *et al.*[11] paid attention to hepatic enlargement during the recovery stage of nutritional dystrophy in Mexican children and they, basing their report upon liver biopsy findings, demonstrated that the hepatic enlargement should be ascribed rather to hydropic degeneration than to fatty infiltration of the hepatic cells.

The children subjected to my own investigation were those from the poorer classes and they showed clear features of avitaminosis B_2, as part manifestations of nutritional dystrophy, and plant cell-like appearance of hepatic cells was found in 22 out of 30 of the biopsied livers.

With respect to the biochemical findings of the children with nutritional dystrophy in the Hirosaki area, a decrease in albumin with an increase in globulin[12], a decrease in riboflavine in the blood[13], and a decrease in protein-bound iodine in sera[14], have been reported from our Department. Recently Morinaga[15] has found a low level of methionine in the blood of these children.

The plant cell-like appearance of hepatic cells which was observed in the children with nutritional dystrophy in Hirosaki area, may be due to a high-carbohydrate-low-protein diet, as in the cases described by Gillman and Gillman[8].

Fatty liver was found in 6 cases, of which 2 showed large fatty drops and were under the age of 12 months. This particular tendency of infancy to develop gross fatty liver was also reported by Gillman and Gillman[8].

Necrotic areas in hepatic lobules were found in 5 cases, of which 4 were under the age of 2 years. Histological findings were those of zonal necrosis[16], which might be caused by chemical, infective and nutritional agents. Himsworth[16] states that, no condition of zonal necrosis, directly due to dietary deficiency, has as yet been distinguished in man, but it is,

as he further states, possible that such exists among races living on low protein diets and will be found if sought. These 5 cases with zonal necrosis of the livers were suffering from no severe infections or of chemical intoxication, so the zonal necrosis of these livers may be due to feeding on a deficient diet, that is, high-carbohydrate-low-protein diet.

Hepatic fibrosis was, according to Himsworth[16], divided into 2 forms, that is, post-necrotic scarring and diffuse fibrosis. The diffuse hepatic fibrosis is the classical feature of 'portal fibrosis'. Himsworth[16] says that in distinguishing diffuse fibrosis from post-necrotic scarring, there are three features that must be stressed in the former. First, the development is insidious, not sudden, Second, frank necrosis does not occur at any stage. Third, the process is uniform and when the lesion is established, every lobule is affected and no normal lobulation can be found. The diffuse fibrosis is seen ; (1) after infiltration of neutral fat, glycogen or lipids ; (2) after repeated frequent attacks of zonal necrosis and (3) following biliary obstruction[16].

In the present investigation diffuse fibrosis, including its early stage, was found in 12 cases of the biopsied livers. These diffuse fibrosis will, as mentioned by Himsworth[16], be caused partly by repeated attacks of zonal necrosis (toxipathic, probably combined with trophopathic hepatitis) and partly by an accumulation of glycogen in the hepatic cells.

Basophilia of the liver parenchyma, which was seen in 10 cases, is related to active protein synthesis[17].

In 1953, Hill *et al.*[18] reported serous hepatosis among Jamaican children who have been fed on high-carbohydrate-low-protein diet. Clinical features of this illness were hepatomegaly with or without ascites, dysproteinemia, a decrease in serum cholin esterase, but rare in signs of vitamin deficiency. The liver biopsy findings of these children showed eosinophilic coagulum in the space of Disse, fibrosis, dilated sinusoid filled with blood and a hydropic appearance of the hepatic cells.

Eosinophlic coagulum in the space of Disse, which was commonly observed in the biopsied livers of serous hepatosis, was found in only 2 cases of our present investigation.

Conclusions

In 30 cases of biopsied liver from children with nutritional dystrophy, plant cell-like appearance of the hepatic cells was found in 22, cloudy swelling in 10 cases, fatty infiltration in 5 cases, diffuse fibrosis in 12 cases, zonal necrosis in 5 cases, basophilia of the hepatic parenchyma in 11 cases, and cosinophilic coagulum in the space of Disse in 2 cases, and a thickning of arterial wall in one case.

I wish to express my heartfelt thanks to Prof. K. Inoue (Kyoto University) for his encouragement. Y. Sato.

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Protein-Bound Iodine Levels in Children with Nutritional Dystrophy

Studies on the Nutrition of Children in Hirosaki Area

(26th Report)

By

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In a preceding paper, Arakawa and Tachibana[1] reported a retardation in osseous development of wrist in the children with nutritional dystrophtrophy and suggested the possibility of a depressed function of the thyroid in these children.

In the present paper, protein-bound iodine levels in sera from the children with nutritional dystrophy in Hirosaki area will be reported.

Experimental

Materials and Method

The children subjected to this investigation were appearently healthy and attending primary school at Miyoshi Village where nutritional dystrophy has been prevalent among the inhabitants. All these children were boys and of the age from 8 to 10 years.

As clinical manifestations of nutritional dystrophy, angular stomatitis, cheilosis, swelling and/or atrophy of lingual papillae and hyperpigmentation of the skin were carefully observed. According to the severity of these clinical signs of nutritional dystrophy, the children were divided into two grups (Groups A and B); Group A consisted of the children with severe symptoms and Group B of those with less severe symptoms.

Protein-bound iodine in sera was determined according to the method described by Shichijo *et al.*[2], which is a slight modification of Barker's original method[3].

Results and Comment

Protein-bound iodine in sera of healthy children (Cf. Table I). Protein-bound iodine was estimated on 8 healthy children and found to be

TABLE I

Protein-Bound Iodine Content in Sera of Healthy Children

No. of case	Name, age and sex (y.m.)			Protein-bound iodine in serum (gamma per cent)
1	Y.N.	6.3.	♀	6.25
2	Y.Y.	7.2.	♀	6.70
3	Y.E.	5.7.	♀	5.95
4	S.T.	6.4.	♂	5.95
5	T.J.	8.7.	♂	4.50
6	S.Y.	8.3.	♂	6.80
7	I.S.	7.2.	♂	5.70
8	T.Y.	8.5.	♂	6.50
				Average 6.04±0.89

6.04±0.89γ% on an average. This value showed a good accordance with that which reported by Sasaki and Arimasu[4], who found to be 6.00±0.6 gamma per cent as an average of 30 cases of healthy children who were attending primary school.

According to Danowsky *et al.*[5], the protein-bound iodine level was reported to be 5.1±0.9 gamma per cent in the healthy children who were of the age ranging from 9 years and 6 months to 10 years and 11 months and were of North European stock.

Relation between protein-bound iodine level and the severity in nutritional dystrophy (Cf. Table II).

Rapport and Curtis[1] described that the exact nature of the protein-bound iodine was unknown, that it was however, at least in part, thyroxine or thyroxine-like, that the protein-bound iodine values above the normal range (from 4 to 8 gamma per cent) were indicative of hyperthyroidism and that those below were, of hypothyroidism.

In our present investigation (Cf. Table III), the protein-bound iodine levels below 4γ% were found in 11 (52.4%) out of 21 cases belonging to Group A and in only 2 (15.0%) out of 14 cases of Group B.

It is therefore concluded that "depressed function of the thyroid" was more frequently encountered in Group A than in Group B.

This finding can be of interest to us, because a requirement in vitamin B is, according to Gillman and Gillman[7], elevated in hypothyroidism associated with pellagra in Africans. On the other hand, decreased concentration of protein-bound iodine was reported in some intances of hypoproteinemia[8].

Peters and Man[8] have shown that the protein-bound iodine in patients with profound hypoalbuminemia is often reduced as low as in patients

TABLE II

Protein-Bound Iodine in Sera of Children with Nutritional Dystrophy

Group	No. of case	Name, age and sex			Protein-bound iodine in serum (gamma per cent)
Group A	1	K.T.	9.11.	♂	2.25
	2	S.Y.	9.4.	"	1.35
	3	S.H.	9.2.	"	3.00
	4	O.K.	8.6.	"	3.50
	5	K.S.	9.10.	"	4.65
	6	O.K.	10.3.	"	3.10
	7	T.Y.	9.1.	"	2.95
	8	K.Y.	10.2.	"	3.50
	9	N.T.	10.4.	"	2.95
	10	N.S.	9.1.	"	1.25
	11	K.T.	9.11.	"	1.60
	12	K.S.	8.4.	"	4.45
	13	K.T.	9.2.	"	13.60
	14	K.S.	10.2.	"	8.80
	15	K.K.	8.9.	"	7.07
	16	O.K.	9.5.	"	5.37
	17	O.T.	10.2.	"	8.25
	18	O.H.	10.2.	"	6.50
	19	O.K.	10.3.	"	6.95
	20	T.K.	10.1.	"	8.55
	21	K.A.	8.5	"	7.60
Group B	22	S.H.	10.1.	"	3.35
	23	S.A.	8.8.	"	1.62
	24	K.T.	10.1.	"	4.65
	25	S.T.	8.9.	"	4.60
	26	T.H.	9.0.	"	4.85
	27	O.K.	10.3.	"	11.04
	28	T.T.	9.2.	"	6.40
	29	M.M.	10.2.	"	5.90
	30	K.I.	9.1.	"	6.55
	31	I.T.	10.1.	"	5.80
	32	T.S.	8.5.	"	6.70
	33	O.S.	9.9.	"	6.25
	34	S.H.	8.4.	"	6.32
	35	O.Z.	9.5.	"	5.15

TABLE III

Protein-Bound Iodine in Sera of Groups A and B

Protein-bound iodine	Group A	Group B	
Below 4 γ %	11 cases (52.4%)	2 cases (15.0%)	14
Above 4.1 γ %	10 cases	12 cases	21
	21	14	35

with myxedema, although there may be no clinical evidences of thyroid deficiency, that an administration of active thyroid substances in dosage adequate for the treatment of myxedema does not raise the protein-bound iodine in these individuals and that injections of enough salt-poor human albumin to raise the serum albumin considerably, does not consistently raise, and more often decreases, the protein-bound iodine.

As already reported by Yoshida[9)10)] profound hypoalbuminemia was found in the children with nutritional dystrophy at Miyoshi Village. In our present investigation, low level of protein-bound iodine was found in the cases with severe nutritional dystrophy. Whether it may taken as an evidence of " depressed function of thyroid " or as a manifestation of hypoalbuminemia remains to be investigated further.

Summary and Conclusions

Low level of protein-bound iodine was found in the children with nutritional dystrophy.

Wether the low level of protein-bound iodine is a manifestation of hypoalbuminemia or to be ascribed to a depressed function of thyroid in the children with nutritional dystrophy remains to be investigated further.

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